Electronic Supplementary Material (ESI) for Reaction Chemistry & Engineering.

Supporting information file

Framework of the kinetic analysis of O_2-dependent oxidative biocatalysts for reaction intensification

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SUPPORTING FIGURES

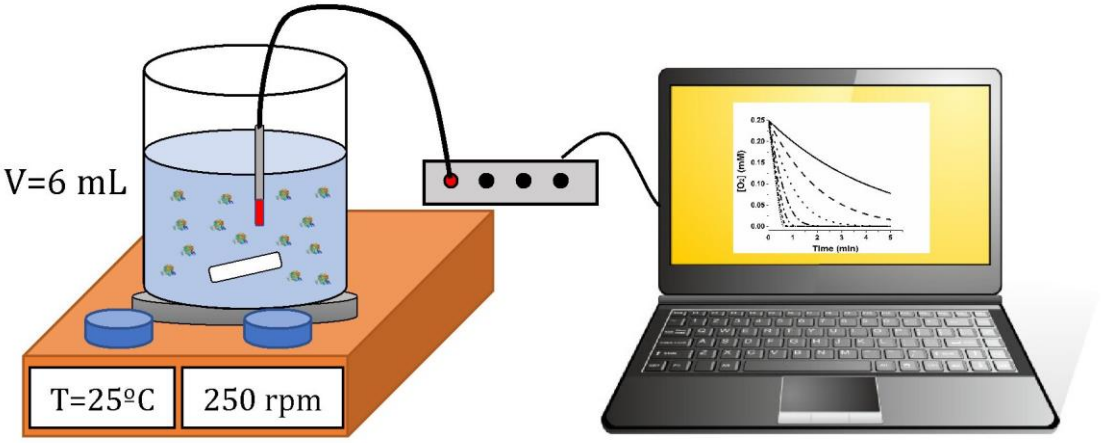

Figure S1. Oxygen consumption time course performed with a fiber-optic oxygen sensor FireSting®-PRO (FSPRO-4) from PyroScience. The experiments were performed in a 6 mL flask controlling the temperature at 25ºC and with magnetically stirring at 250 rpm.

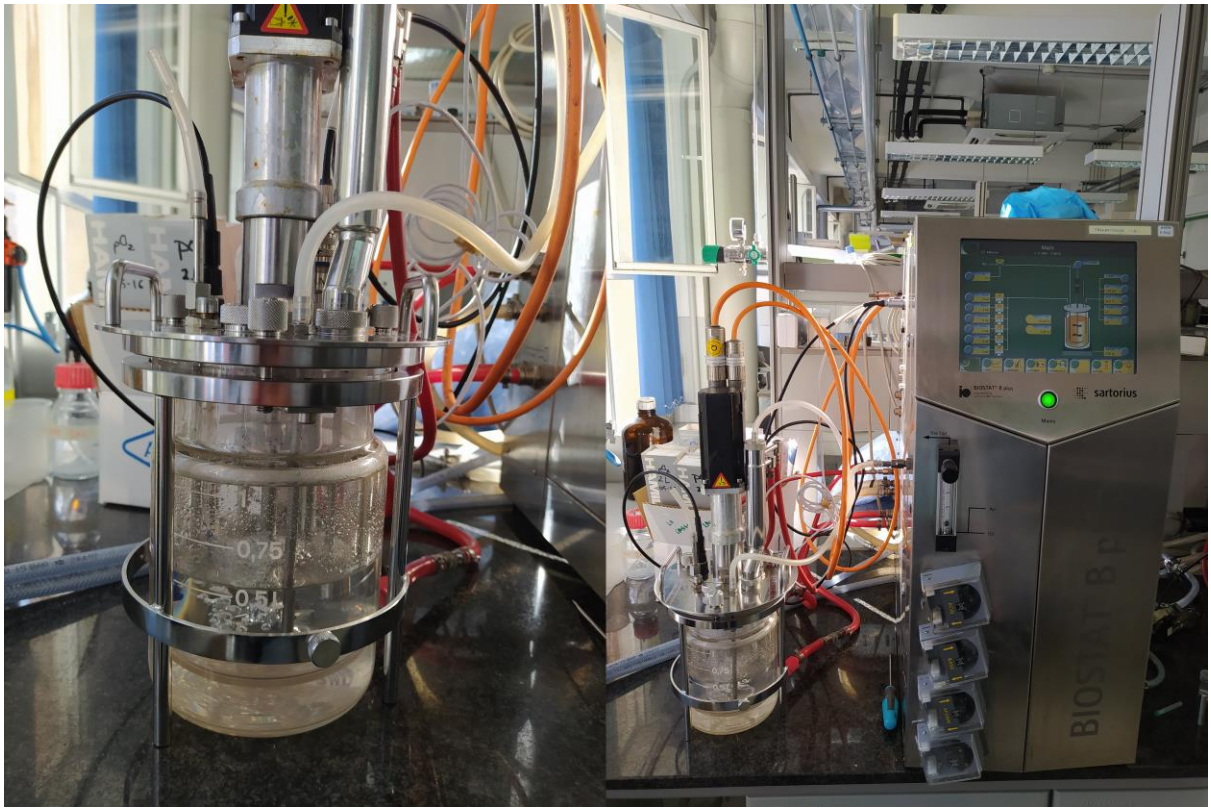

Figure S2. Stirred tank reactor of 1 L (Biostat B Plus) from Sartorius AG where the experiments at medium-scale were performed. The reactor was equipped with a sintered metal sparger and a Rushton turbine for mixing. Oxygen and pH were measured with probes from Hamilton Company.

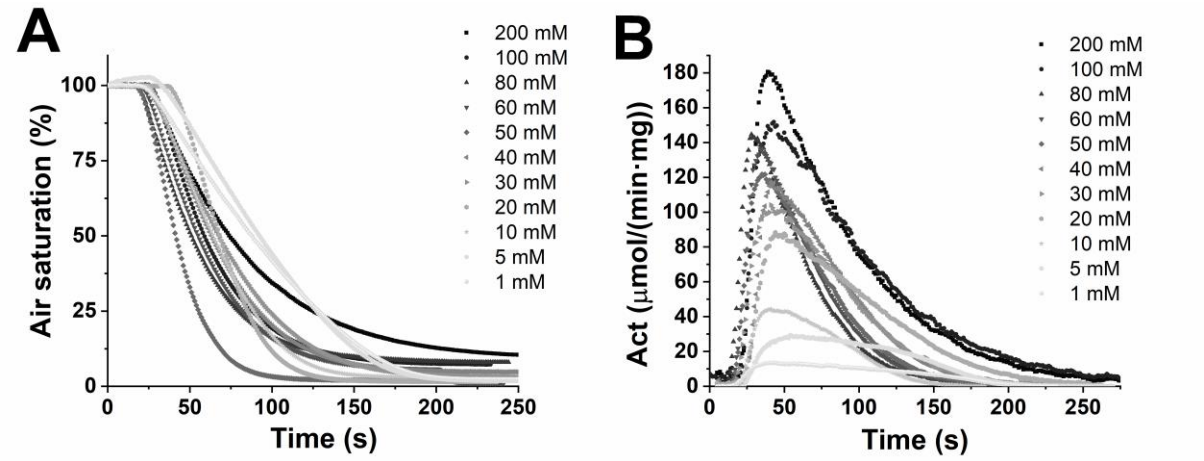

Figure S3. Representative set of oxygen time courses upon variation of main substrate concentration for GOX. Panel A: Air saturation time course, Panel B: Specific reaction rate (enzyme activity) time course. Data in Panel B are obtained by finite incremental differentiation of Panel A data. Concentration of enzyme, E, was adjusted to have a suitable number of data points (E was increased at low activities and E was decreased at high activities; [E]=0.0015-0.003 mg/mL). $[O_2]$ = 0.25 mM at 100 % air saturation conditions and 25 ºC.

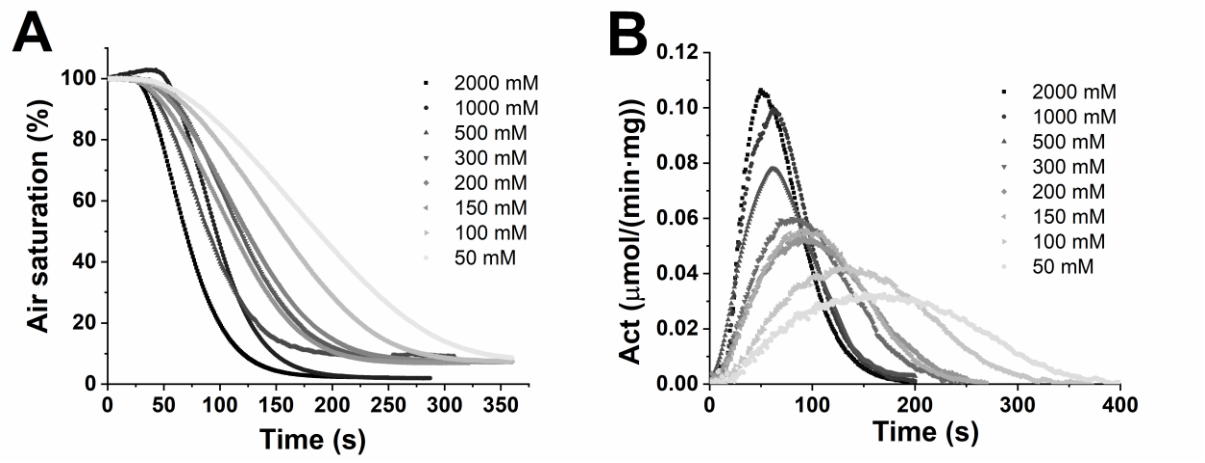

Figure S4. Representative set of oxygen time courses upon variation of main substrate concentration for GalOx with HRP as activator. Panel A: Air saturation time course, Panel B: Specific reaction rate (enzyme activity) time course. Data in Panel B are obtained by finite incremental differentiation of Panel A data. Concentration of enzyme, E, was adjusted to have a suitable number of data points (E was increased at low activities and E was decreased at high activities; [E]=5-10 mg/mL). [O_2] = 0.25 mM at 100 % air saturation conditions and 25 ºC.

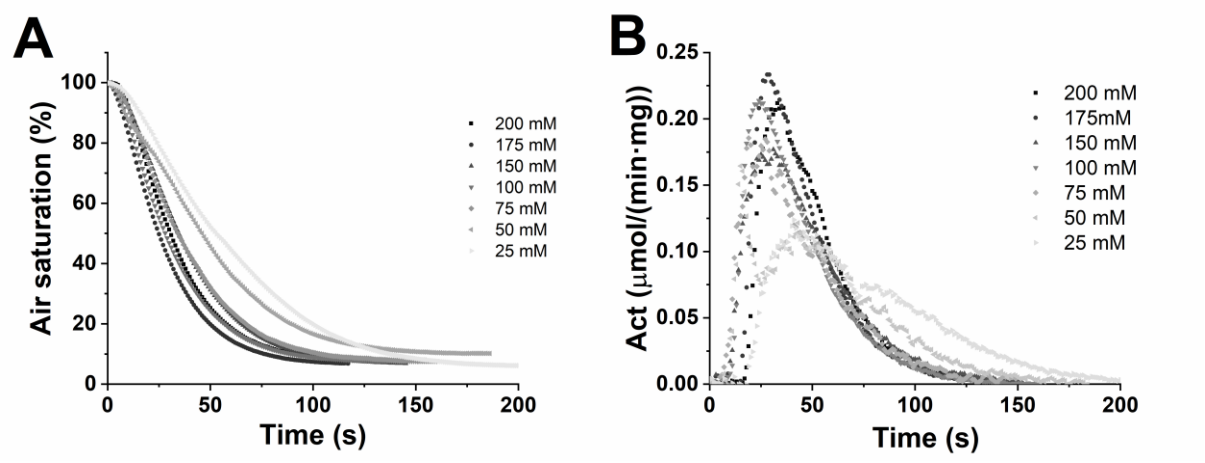

Figure S5. Representative set of oxygen time courses upon variation of main substrate concentration for GalOx with $K_3Fe(CN)_6$ as activator. Panel A: Air saturation time course, Panel B: Specific reaction rate (enzyme activity) time course. Data in Panel B are obtained by finite incremental differentiation of Panel A data. Concentration of enzyme, E, was adjusted to have a suitable number of data points (E was increased at low activities and E was decreased at high activities; [E]=0.5-0.75 mg/mL). $[O_2]$ = 0.25 mM at 100 % air saturation conditions and 25 ºC.

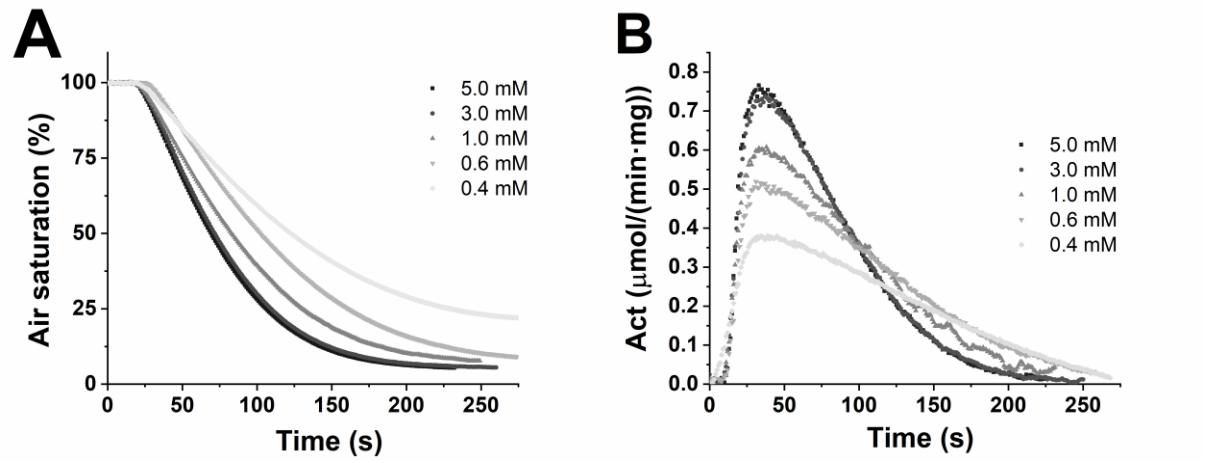

Figure S6. Representative set of oxygen time courses upon variation of main substrate concentration for Laccase. Panel A: Air saturation time course, Panel B: Specific reaction rate (enzyme activity) time course. Data in Panel B are obtained by finite incremental differentiation of Panel A data. Concentration of enzyme, E, was adjusted to have a suitable number of data points, [E]=0.35 mg/mL. $[O_2]$ = 0.25 mM at 100 % air saturation conditions and 25 ºC.

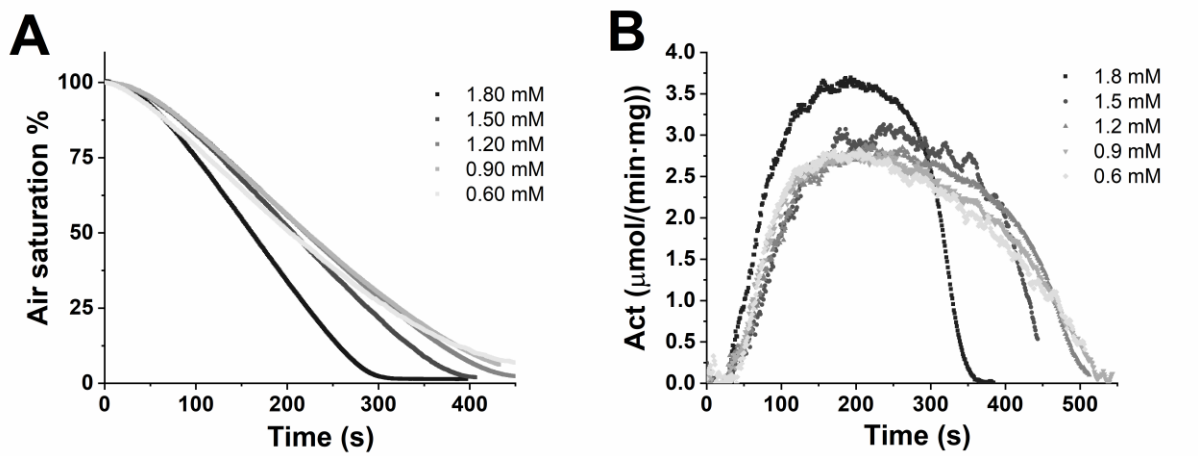

Figure S7. Representative set of oxygen time courses upon variation of main substrate concentration for Tyrosinase. Panel A: Air saturation time course, Panel B: Specific reaction rate (enzyme activity) time course. Data in Panel B are obtained by finite incremental differentiation of Panel A data. Concentration of enzyme, E, was adjusted to have a suitable number of data points (E was increased at low activities and E was decreased at high activities; [E]=0.10-0.16 mg/mL). [O_2] = 0.25 mM at 100 % air saturation conditions and 25 ºC.

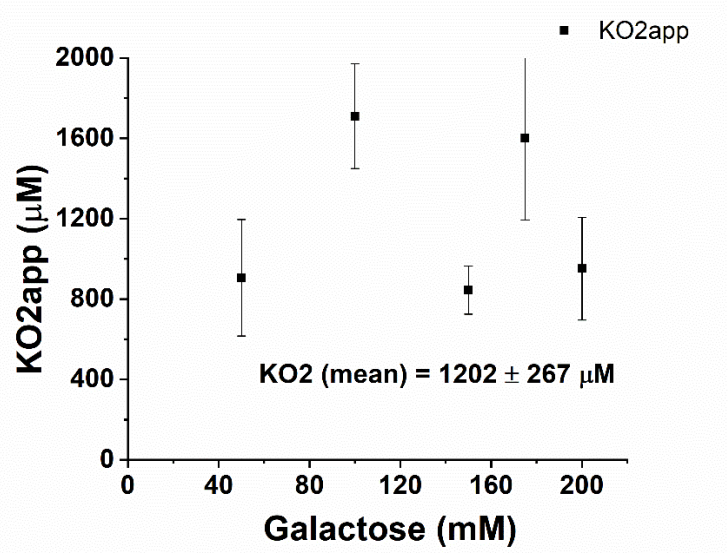

Figure S8. Plot of differential analysis of [O_2] for GalOx with $K_3Fe(CN)_6$ as activator, apparent kinetic constant for oxygen (KO_{2app}) varying the galactose concentration. [E]=1.6 mg/mL.

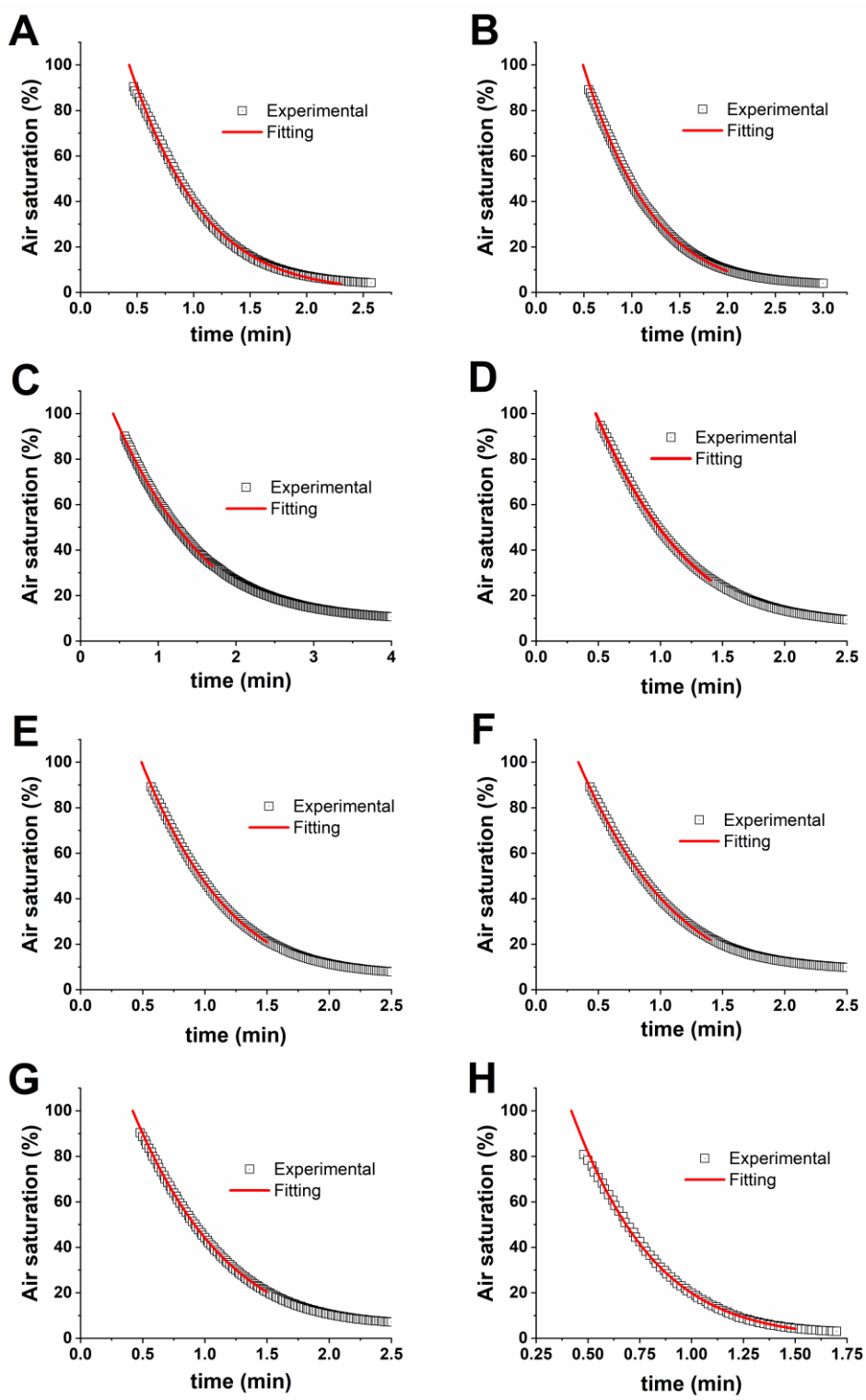

Figure S9. Single response integral analysis with BM for GOX. Panel A: 2000 mM of glucose, Panel B: 400 mM of glucose, Panel C: 200 mM of glucose, Panel D: 100 mM of glucose, Panel E: 90 mM of glucose, Panel F: 80 mM of glucose, Panel G: 60 mM of glucose, Panel H: 50 mM of glucose. Experimental data (square dots), simulated data (red line).

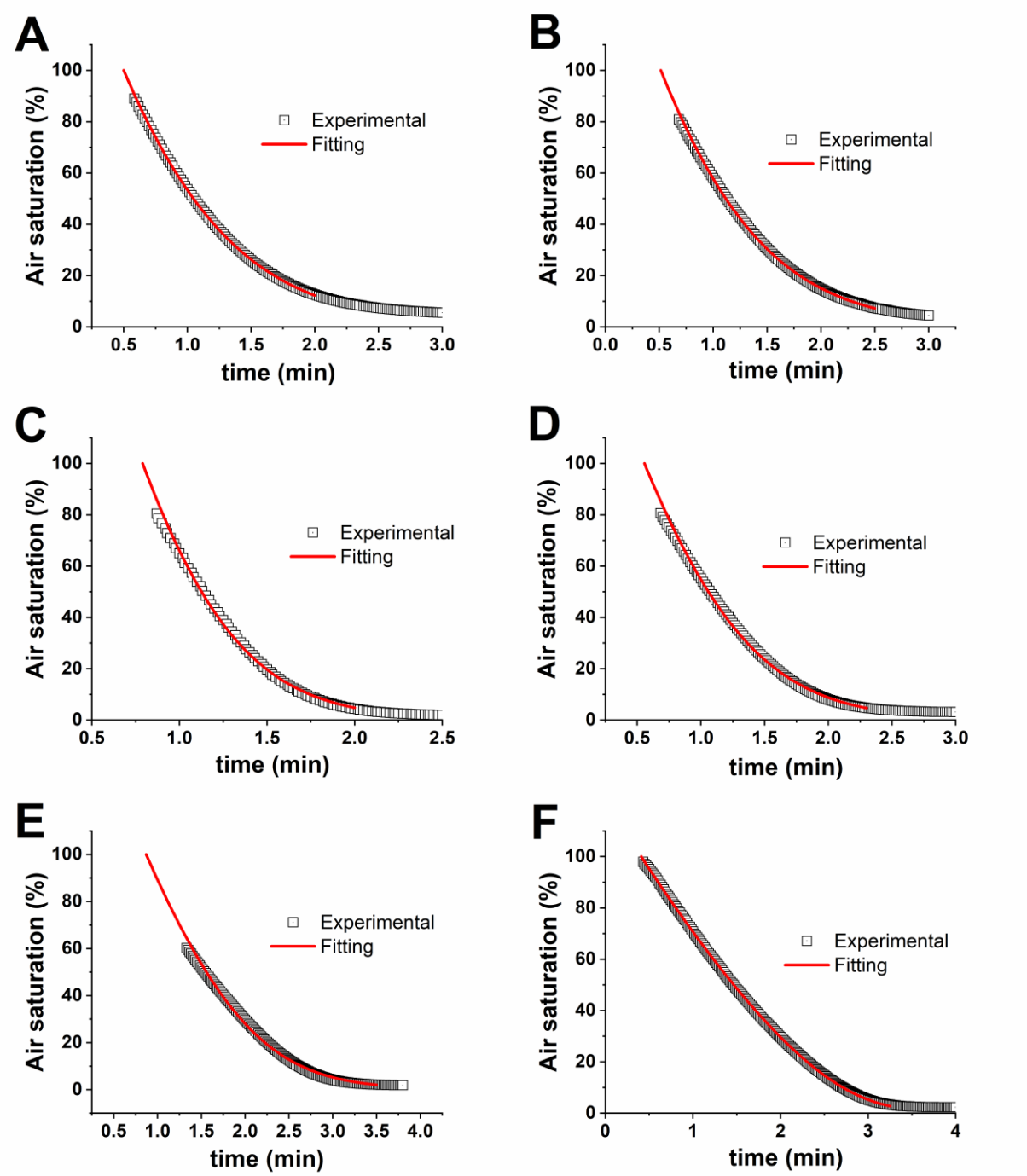

Figure S10. Single response integral analysis with BM for GOX. Panel A: 40 mM of glucose, Panel B: 30 mM of glucose, Panel C: 20 mM of glucose, Panel D: 10 mM of glucose, Panel E: 5 mM of glucose, Panel F: 1 mM of glucose. Experimental data (square dots), simulated data (red line).

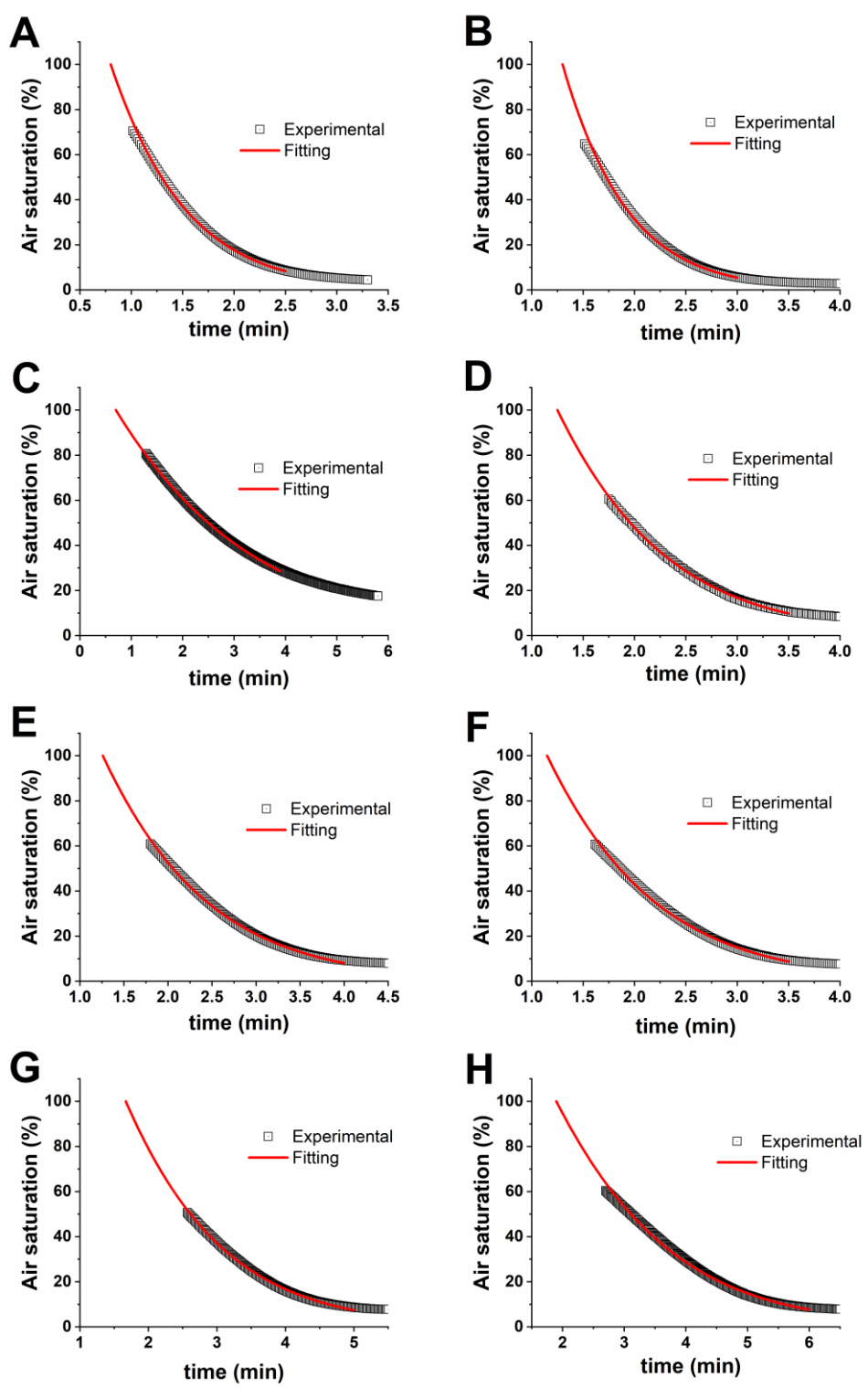

Figure S11. Single response integral analysis with BM for GalOx with HRP as activator. Panel A: 2000 mM of galactose, Panel B: 1000 mM of galactose, Panel C: 500 mM of galactose, Panel D: 300 mM of galactose, Panel E: 200 mM of galactose, Panel F: 150 mM of galactose, Panel G: 100 mM of galactose, Panel H: 50 mM of galactose. Experimental data (square dots), simulated data (red line).

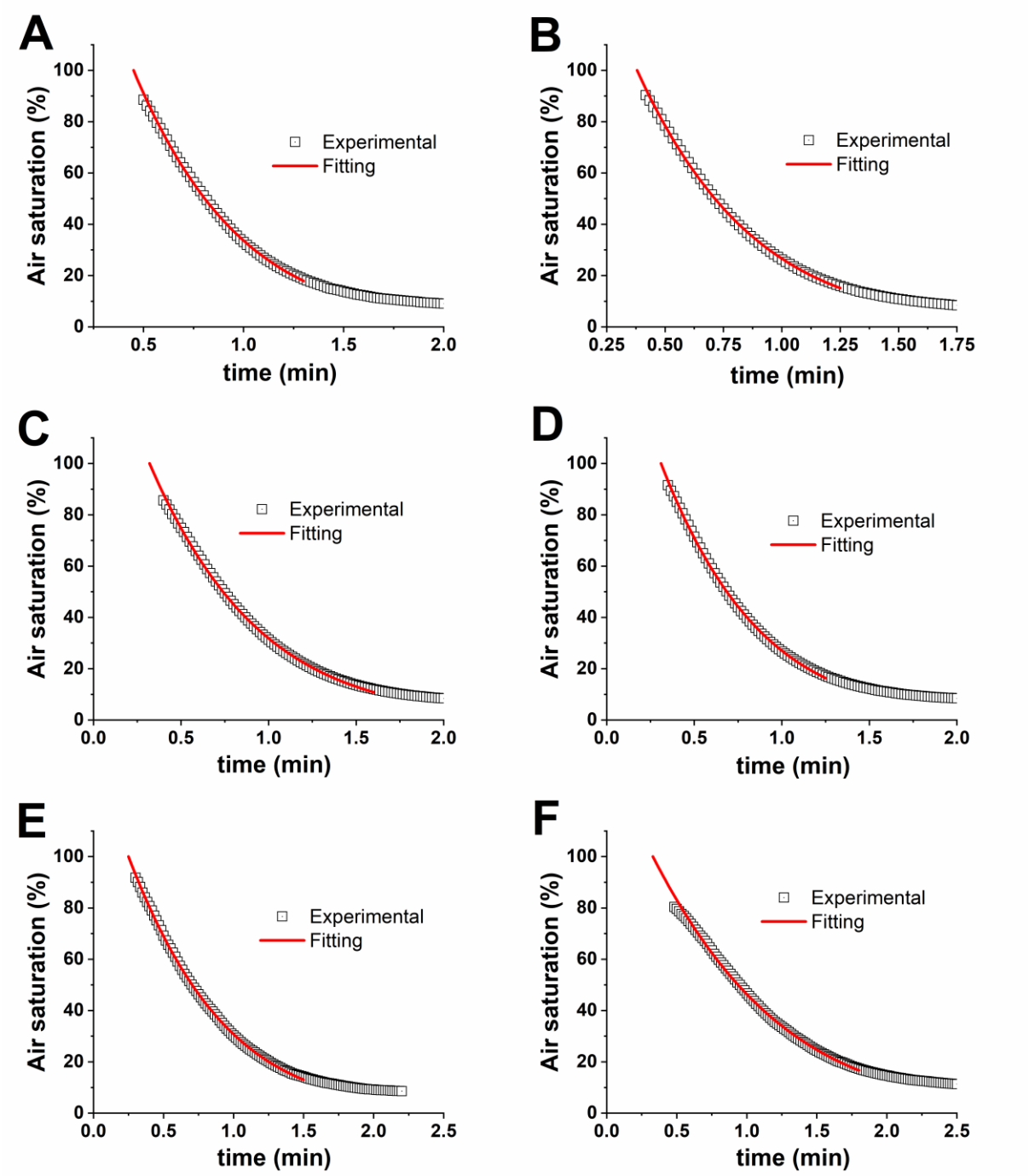

Figure S12. Single response integral analysis with BM for GalOx with $K_3Fe(CN)_6$ as activator. Panel A: 200 mM of galactose, Panel B: 175 mM of galactose, Panel C: 150 mM of galactose, Panel D: 100 mM of galactose, Panel E: 75 mM of galactose, Panel F: 50 mM of galactose. Experimental data (square dots), simulated data (red line).

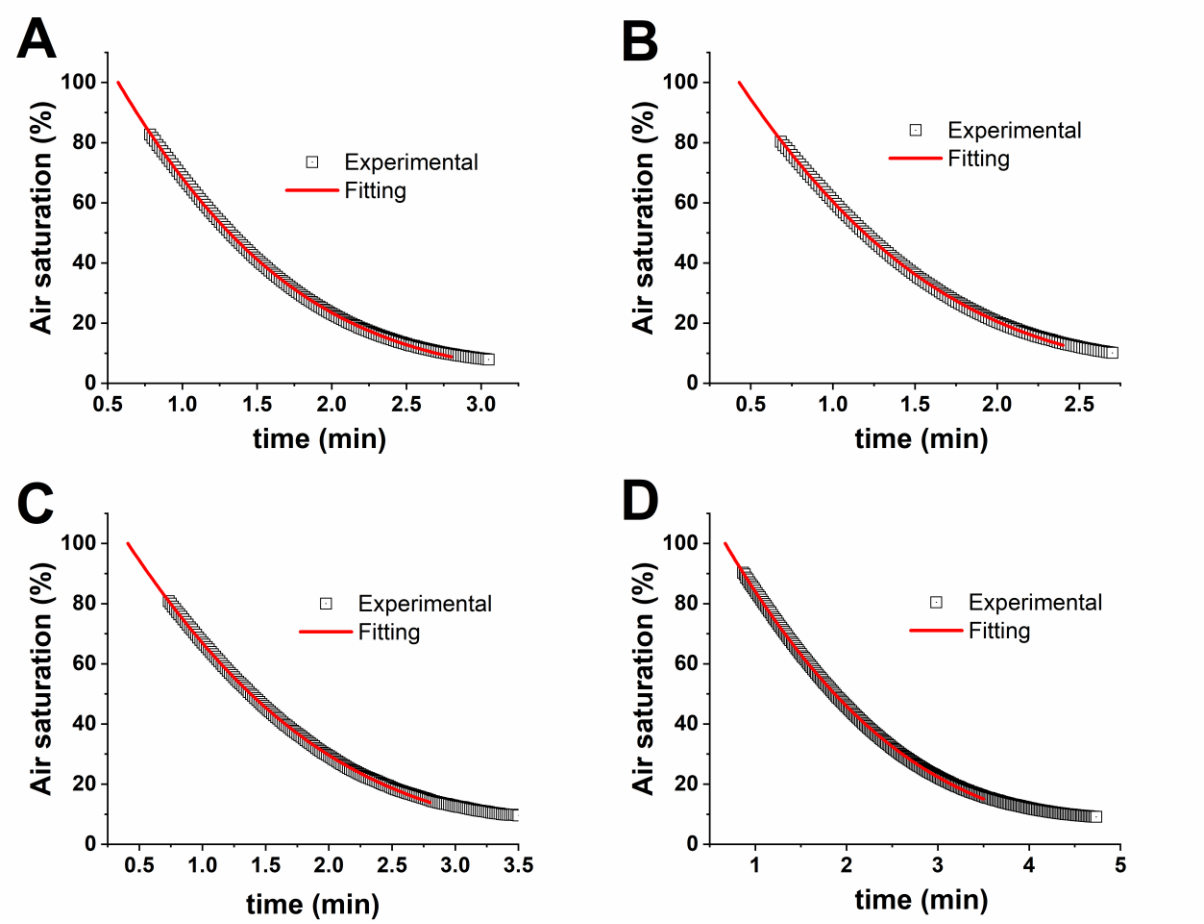

Figure S13. Single response integral analysis with BM for laccase. Panel A: 5 mM of catechol, Panel B: 3 mM of catechol, Panel C: 1 mM of catechol, Panel D: 0.6 mM of catechol. Experimental data (square dots), simulated data (red line).

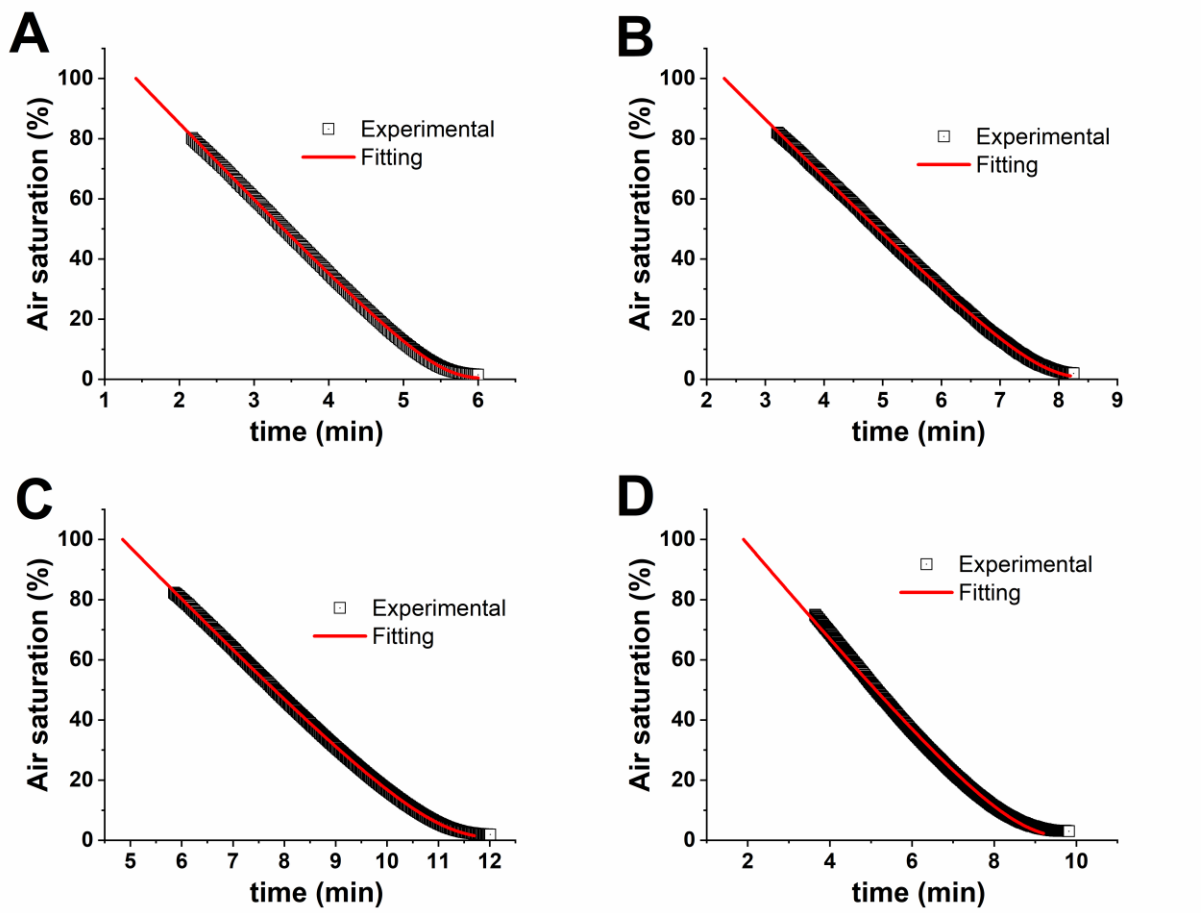

Figure S14. Single response integral analysis with BM for tyrosinase. Panel A: 1.8 mM of tyrosine, Panel B: 1.5 mM of tyrosine, Panel C: 1.2 mM of tyrosine, Panel D: 0.9 mM of tyrosine. Experimental data (square dots), simulated data (red line).

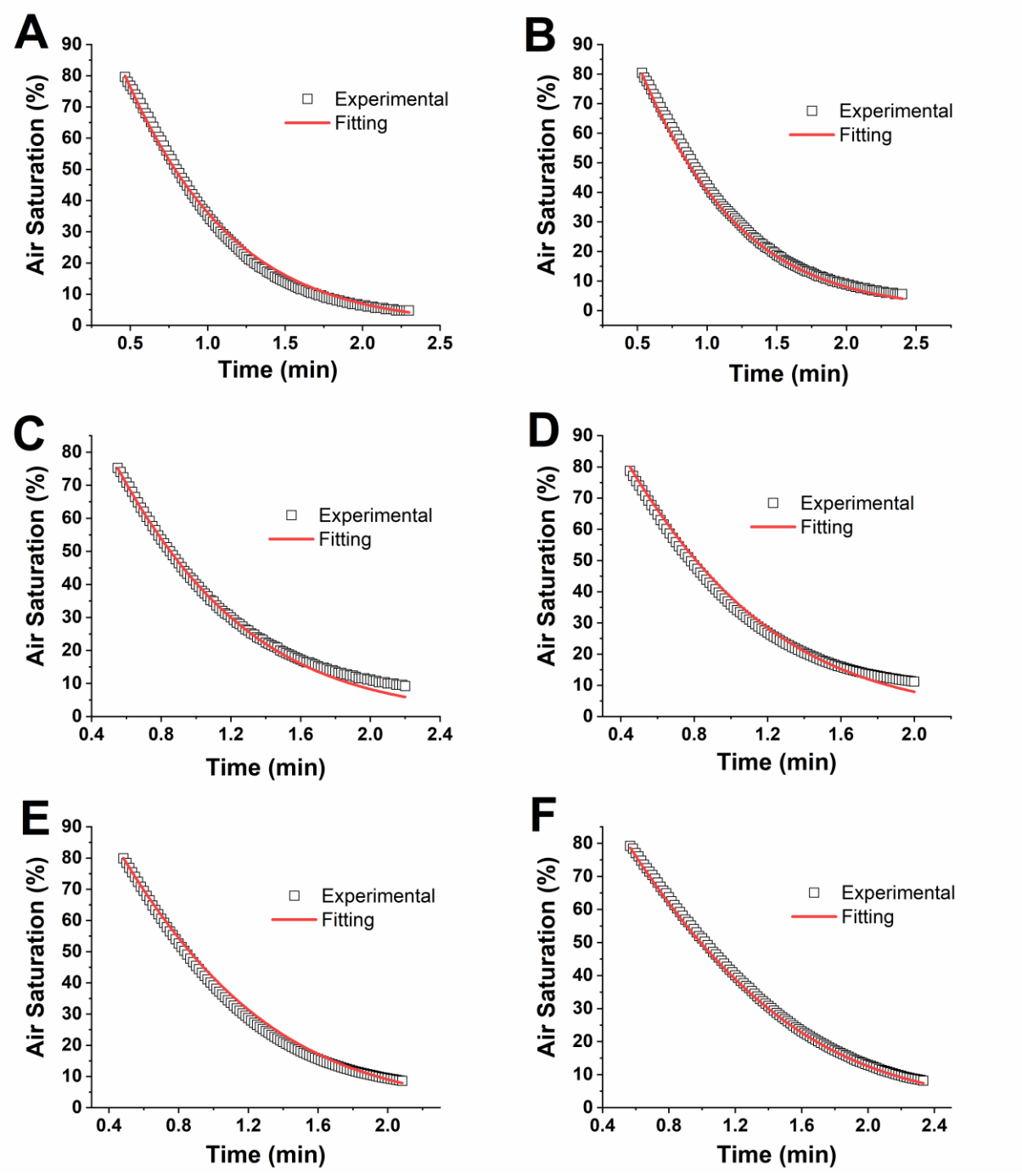

Figure S15. Multiple response integral analysis with Aspen for GOX. Panel A: 2000 mM of glucose, Panel B: 400 mM of glucose, Panel C: 100 mM of glucose, Panel D: 75 mM of glucose, Panel E: 50 mM of glucose, Panel F: 25 mM of glucose. Experimental data (square dots), simulated data (red line).

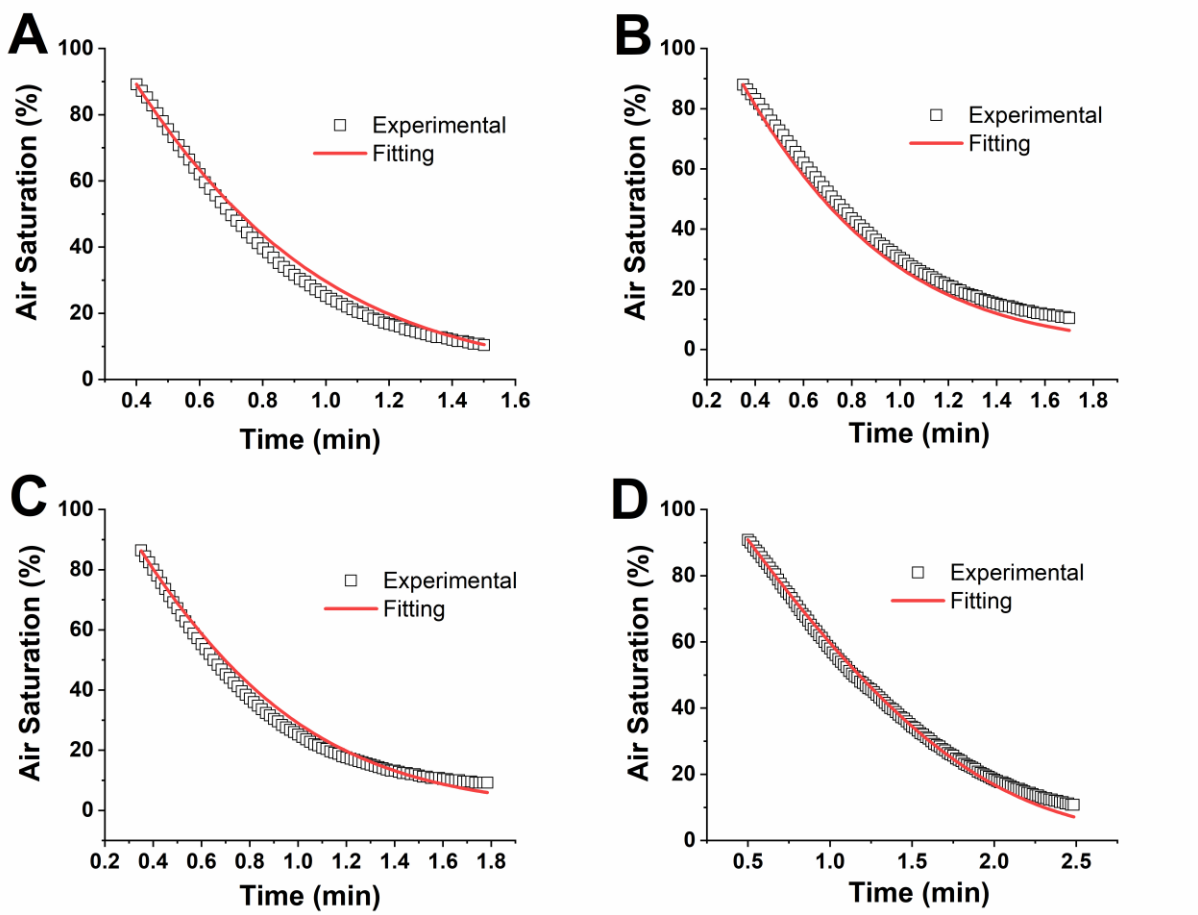

Figure S16. Multiple response integral analysis with Aspen for GalOx with $K_3Fe(CN)_6$. Panel A: 175 mM of galactose, Panel B: 150 mM of galactose, Panel C: 100 mM of galactose, Panel D: 25 mM of galactose. Experimental data (square dots), simulated data (red line).

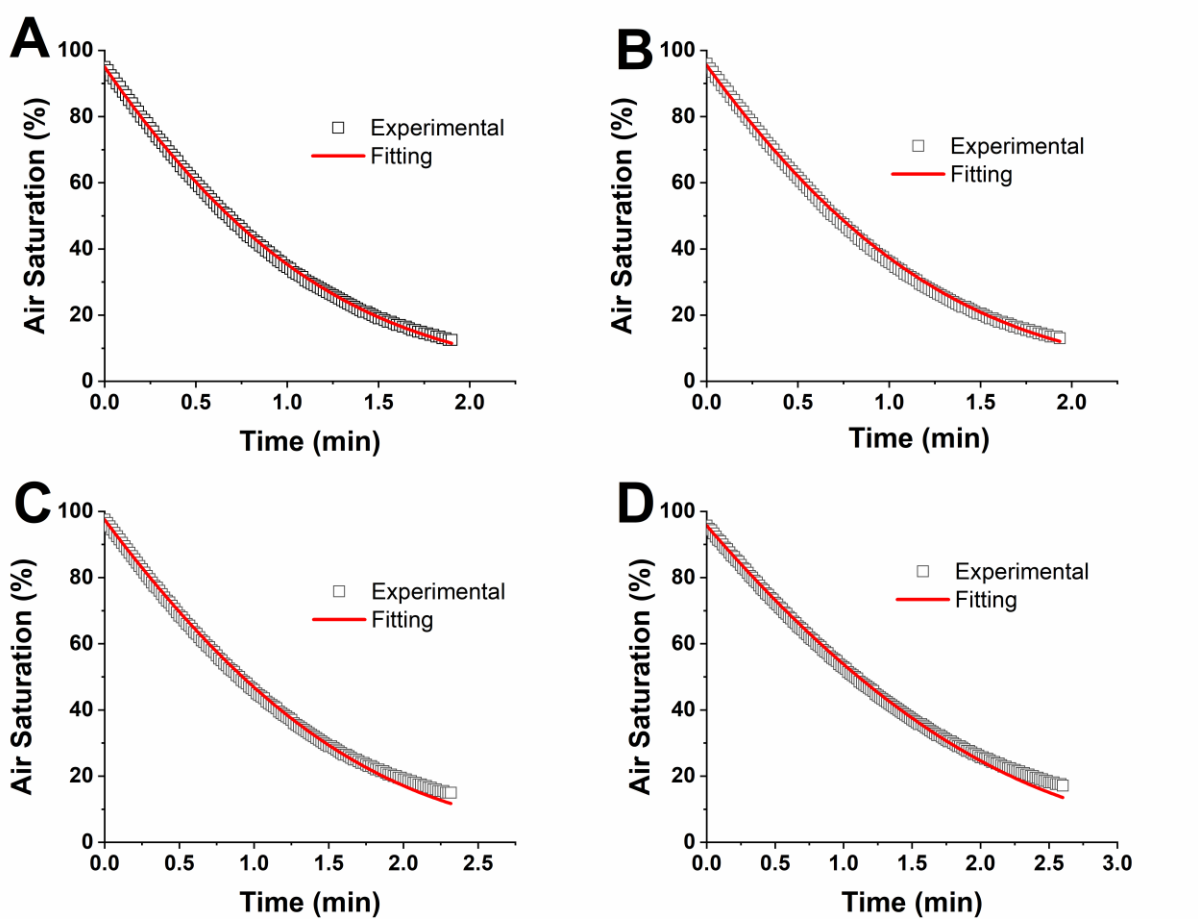

Figure S17. Multiple response integral analysis with Aspen for laccase. Panel A: 5.0 mM of catechol, Panel B: 3.0 mM of catechol, Panel C: 1.0 mM of catechol, Panel D: 0.6 mM of catechol. Experimental data (square dots), simulated data (red line).

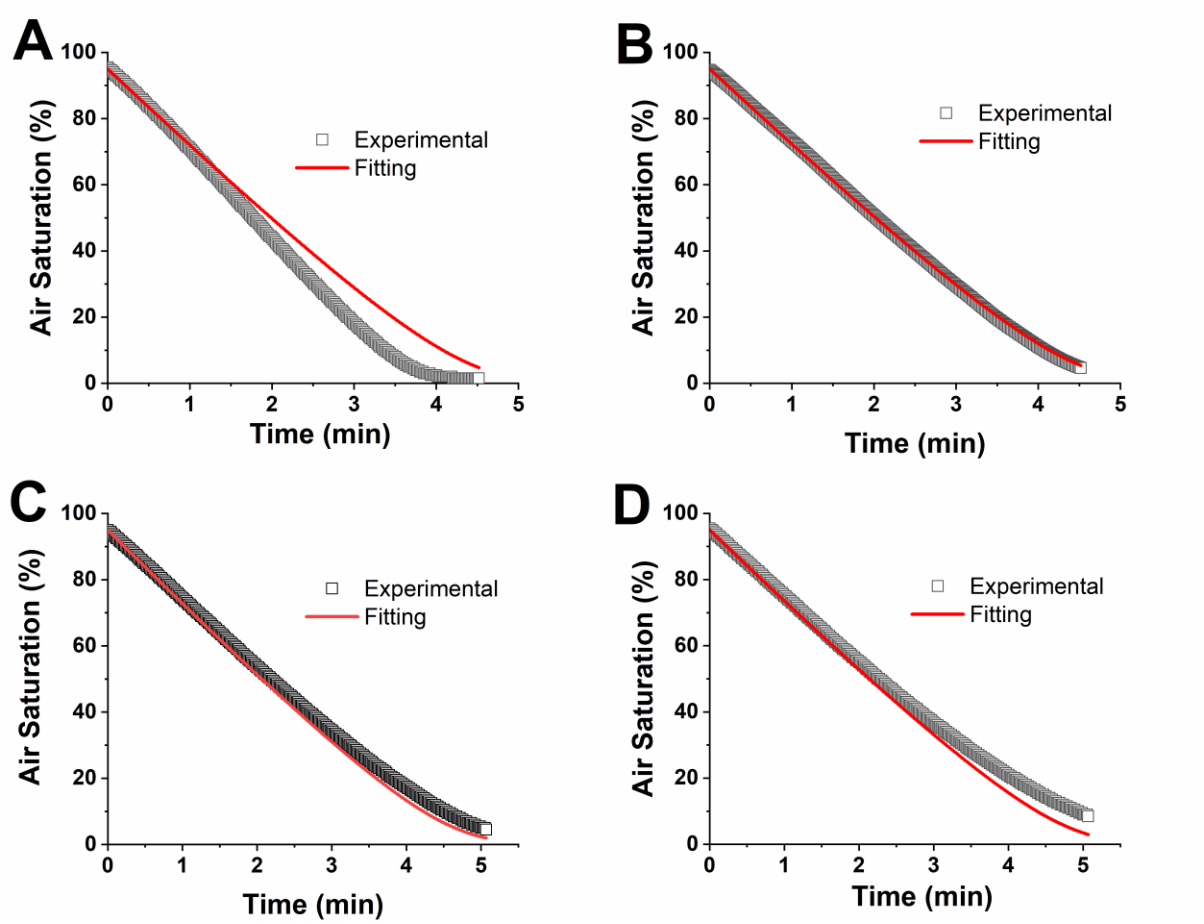

Figure S18. Multiple response integral analysis with Aspen for tyrosinase. Panel A: 1.8 mM of tyrosine, Panel B: 1.5 mM of tyrosine, Panel C: 1.2 mM of tyrosine, Panel D: 0.9 mM of tyrosine. Experimental data (square dots), simulated data (red line).

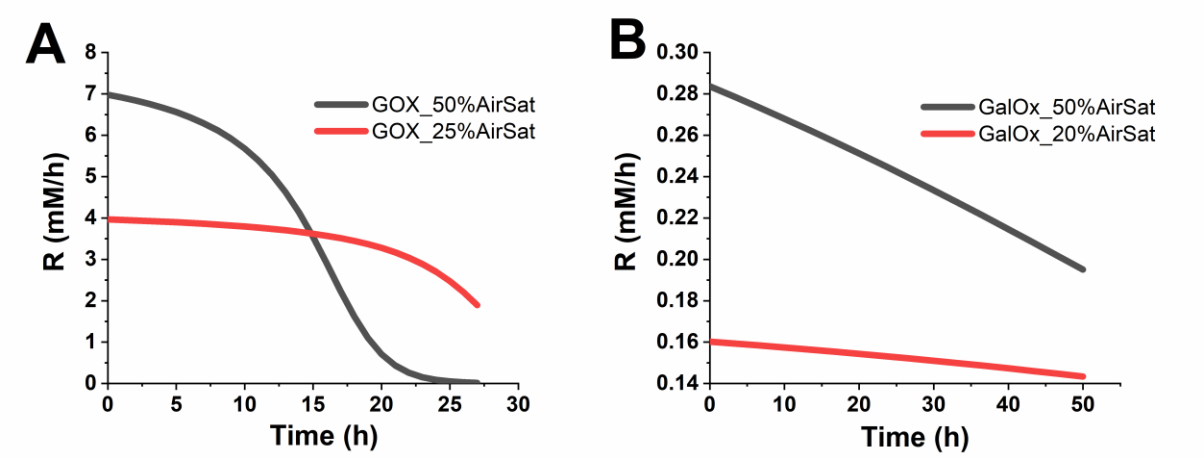

Figure S19. Simulation of reaction rate vs time for the medium-scale experiments in 500 mL. Panel A: GOX, [Glucose$_0$]=100 mM, [E$_0$]=0.0015 mg/mL, Act standard=130 μmol/(min·mg), 50% air saturation (black line), 25% air saturation (red line). Panel B: [Galactose$_0$]=20 mM, [E$_0$]=0.05 mg/mL, Act standard=0.13 μmol/(min·mg), 50% air saturation (black line), 20% air saturation (red line).

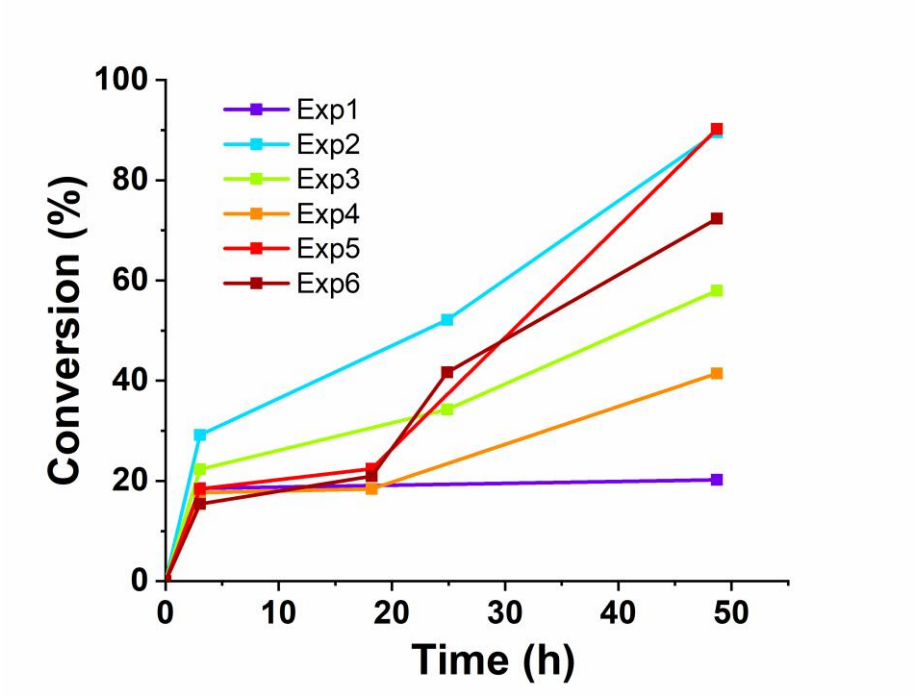

Figure S20. Conversion of galactose vs time experiments for GalOx with surface aeration. Exp1: 4.6 mg/mL enzyme, 0.09 mg/mL HRP, no catalase. Exp2: 4.6 mg/mL enzyme, 0.18 mg/mL HRP, 0.09 mg/mL catalase. Exp3: 4.2 mg/mL enzyme, 0.17 mg/mL HRP, 0.84 mg/mL catalase. Exp4: 4.5 mg/mL enzyme, 0.18 mg/mL $K_3Fe(CN)_6$, no catalase. Exp5: 4.5 mg/mL enzyme, 0.18 mg/mL $K_3Fe(CN)_6$, 0.09 mg/mL catalase. Exp6: 2.5 mg/mL enzyme, 0.17 mg/mL $K_3Fe(CN)_6$, 0.17 mg/mL catalase. $[Galactose]_0$= 20 mM.

Supporting Tables.

Table S1. Summary of the results of the single response integral fitting with BM for GOX.

Glucose (mM)	***KO*$_2$ (µM)**	***K*$_S$ (mM)**	***Act*$_{max}$ (µmol/(min·mg))**	***[E]* (mg/mL)**	**SQR**	**RMSE**	**F Fischer**	**AICc**
2000	867	111	816	0.0020	145	0.97	166836	-6
400	838	108	724	0.0020	64	0.59	499903	-185
200	872	112	588	0.0015	140	0.682	583019	-226
100	815	106	686	0.0020	20.8	0.336	5565343	-397
90	860	110	770	0.0020	18.0	0.266	2440818	-671
80	776	101	873	0.0015	16.5	0.396	989673	-191
60	1007	136	850	0.0015	19.0	0.432	515175	-167
50	940	129	604	0.0050	110	1.04	49305	12
40	745	94	815	0.0015	22.9	0.383	738459	-295
30	701	114	723	0.0015	55	0.57	355803	-183
20	618	81.6	622	0.0030	245	1.54	27533	93
10	632	87	702	0.0020	228	1.24	63336	68
5	615	85.4	817	0.0015	347	1.23	93501	99
1	701	94	770	0.0030	9.3	0.197	5150069	-773

Table S2. Average of the results of the single response integral fitting with BM for GOX.

KO_2 (µM)	K_S (mM)	Act_{max} (µmol/(min·mg))	SQR	RMSE	F Fischer	AICc
785 ± 122	105 ± 15.8	740 ± 91.7	103	0.71	1231341	-201.6

Table S3. Summary of the results of the single response integral fitting with BM for GalOx with HRP.

Galactose (mM)	***KO*$_2$ (µM)**	***K*$_S$ (mM)**	***Act*$_{max}$ (µmol/(min·mg))**	***[E]* (mg/mL)**	**SQR**	**RMSE**	**F Fischer**	**AICc**
2000	1590	293	0.243	5	113	0.79	240120	-79
1000	1550	288	0.277	5	205	1.274	80730	65
500	1770	324	0.074	5	31	0.333	5177954	-614
300	1380	239	0.306	5	40	0.45	945921	-321
200	1660	281	0.354	5	54	0.596	420167	-152
150	1880	314	0.414	5	66	0.713	261192	-84
100	1820	305	0.305	5	45	0.507	592528	-232
50	1460	251	0.203	5	162	0.9	157837	-20

Table S4. Average of the results of the single response integral fitting with BM for GalOx with HRP.

KO_2 (µM)	K_S (mM)	Act_{max} (µmol/(min·mg))	SQR	RMSE	F Fischer	AICc
1640 ± 175	287 ± 29.5	0.272 ± 0.103	89	0.70	984556	-180

Table S5. Summary of the results of the single response integral fitting with BM for GalOx with $K_3Fe(CN)_6$.

Galactose (mM)	***KO*$_2$ (µM)**	***K*$_S$ (mM)**	***Act*$_{max}$ (µmol/(min·mg))**	***[E]* (mg/mL)**	**SQR**	**RMSE**	**F Fischer**	**AICc**
200	1630	314	3.167	1.13	24.0	0.633	179461	-50.87
175	1730	330	3.58	1.13	19.3	0.572	207052	-61.9
150	1910	361	3.13	1.13	26.1	0.62	210390	-59.2
100	2090	392	3.87	1.13	13.7	0.467	347834	-91.9
75	1450	283	2.32	1.13	13.5	0.421	502383	-127.5
50	1570	304	2.91	1.13	84	0.94	140342	-8
25	1410	277	2.46	1.13	35.5	0.500	569197	-192.9

Table S6. Average of the results of the single response integral fitting with BM for GalOx with $K_3Fe(CN)_6$.

KO_2 (µM)	K_S (mM)	Act_{max} (µmol/(min·mg))	SQR	RMSE	F Fischer	AICc
1680 ± 184	323 ± 41.7	3.06 ± 0.559	31	0.59	308094	-84.7

Table S7. Summary of the results of the single response integral fitting with BM for laccase.

Catechol (mM)	***KO*$_2$ (µM)**	***K*$_S$ (mM)**	***Act*$_{max}$ (µmol/(min·mg))**	***[E]* (mg/mL)**	**SQR**	**RMSE**	**F Fischer**	**AICc**
5.0	367	1.25	2.81	0.350	15.2	0.332	1355386	-300
3.0	347	1.22	2.66	0.350	6.0	0.227	2893892	-343
1.0	352	1.21	2.19	0.350	12.7	0.295	2201602	-352
0.6	327	1.19	1.72	0.350	18.7	0.315	2381384	-430

Table S8. Average of the results of the single response integral fitting with BM for laccase.

KO_2 (µM)	K_S (mM)	Act_{max} (µmol/(min·mg))	SQR	RMSE	F Fischer	AICc
348 ± 50	1.22 ± 0.183	2.35 ± 0.49	13.1	0.292	2208066	-357

Table S9. Summary of the results of the single response integral fitting with BM for tyrosinase.

Tyrosine (mM)	***KO*$_2$ (µM)**	***K*$_S$ (mM)**	***Act*$_{max}$ (µmol/(min·mg))**	***[E]* (mg/mL)**	**SQR**	**RMSE**	**F Fischer**	**AICc**
1.8	10.7	0.147	4.20	0.016	38	0.38	2480520	-486
1.5	11.1	0.148	3.69	0.013	31	0.31	5083505	-769
1.2	16.7	0.150	4.40	0.0105	25	0.251	8879887	-1105
0.9	17.5	0.151	4.18	0.01	401	0.96	653753	-32

Table S10. Average of the results of the single response integral fitting with BM for tyrosinase.

KO_2 (µM)	K_S (mM)	Act_{max} (µmol/(min·mg))	SQR	RMSE	F Fischer	AICc
14.0 ± 3.60	0.149 ± 0.0155	4.12 ± 0.304	124	0.48	4274416	-598

Table S11. Summary of the single response integral analysis with BM of each enzyme:

	GOX	**GalOx_HRP**	**GalOx_ $K_3Fe(CN)_6$**	**Laccase**	**Tyrosinase**
***KO_2* (µM)**	785 ± 122	1640 ± 175	1680 ± 184	348 ± 52.0	14.0 ± 3.60
***K_S* (mM)**	105 ± 15.8	287 ± 29.5	323 ± 41.7	1.22 ± 0.183	0.149 ± 0.016
***Act_{max}* (µmol/(min·mg))**	740 ± 91.7	0.272 ± 0.103	3.062 ± 0.559	2.35 ± 0.49	4.12 ± 0.304
SQR	103	89	31	13.1	124
RMSE	0.71	0.70	0.59	0.292	0.48
F Fischer	1231341	984556	308094	2208066	4274416
AICc	-202	-180	-84.7	-357	-598

Table S12. Summary of the multiple response integral analysis with Aspen for GOX.

	KO_2 (µM)	K_S (mM)	Act_{max} (µmol/(min·mg))	SQR	RMSE	F Fischer	AICc
GOX	741 ± 3.0	53.3 ± 1.5	740 ± 91.7	0.070	0.025	15625	-775

Table S13. Summary of the multiple response integral analysis with Aspen for GalOx with $K_3Fe(CN)_6$.

	KO_2 (µM)	K_S (mM)	Act_{max} (µmol/(min·mg))	SQR	RMSE	F Fischer	AICs
GalOx	2070 ± 18	460 ± 7.7	3.06 ± 0.56	0.161	0.036	151947	-667

Table S14. Summary of the multiple response integral analysis with Aspen for laccase.

	KO_2 (µM)	K_S (mM)	Act_{max} (µmol/(min·mg))	SQR	RMSE	F Fischer	AICs
Laccase	305 ± 9.0	1.22 ± 0.18	1.10 ± 0.01	0.006	0.006	1624768	-1415

Table S15. Summary of the multiple response integral analysis with Aspen for tyrosinase.

	KO_2 (µM)	K_S (mM)	Act_{max} (µmol/(min·mg))	SQR	RMSE	F Fischer	AICs
Tyrosinase	15.9 ± 1.5	0.15 ± 0.016	5.43 ± 0.064	4.044	0.099	23822269	-1878

Table S16. Summary of the multiple response integral analysis with Aspen of each enzyme:

	GOX	**GalOx**	**Laccase**	**Tyrosinase**
KO_2 **(µM)**	741 ± 3.0	2070 ± 18.0	305 ± 9.0	16.0 ± 1.47
K_S **(mM)**	53.3 ± 1.5	460 ± 7.7	1.22 ± 0.18	0.150 ± 0.002
Act_{max} **(µmol/(min·mg))**	740 ± 91.7	3.06 ± 0.56	1.10 ± 0.01	5.43 ± 0.064
SQR	0.0702	0.161	0.00599	4.04
RMSE	0.0246	0.0360	0.00582	0.099
F Fischer	15625	151947	1624768	23822269
AICc	-775	-667	-1415	-1878

Table S17. Values of the kinetic parameters proposed of the Ping-Pong model for each enzyme.

	GOX	**GalOx**	**Laccase**	**Tyrosinase**
KO_2 **(µM)**	785 ± 122	1640 ± 175	305 ± 9.0	16.0 ± 1.47
K_S **(mM)**	105 ± 15.8	287 ± 29.5	1.22 ± 0.18	0.150 ± 0.002
Act_{max} **(µmol/(min·mg))**	740 ± 91.7	0.272 ± 0.10	1.10 ± 0.01	5.43 ± 0.064