Supplementary Information

Comparative Study of Degradation of Reactive Dyes and Decolorization and Detoxification in Aqueous Solution Applying DyP Peroxidases Isolated from *Saccharomonospora viridis* (*Svi*DyP) and *Thermobifida fusca* (*Tfu*Dyp)

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Table S1. Results for degradation reactions applying *Svi*DyP and *Tfu*DyP in pH 3

entry	Dye	Enzyme	E in 30 min (pH 3)[a,b] / %	E in 12 h (pH 3)[a,b] / %
1	Reactive Green 19	control[c]	0 ± 0	3 ± 0.8
		*Tfu*DyP	43 ± 0.8	17 ± 5.7
		*Svi*DyP	67 ± 2.4	100 ± 0
2	Reactive Blue 198	control[c]	0 ± 2	0 ± 0
		*Tfu*DyP	100 ± 0	76 ± 2.5
		*Svi*DyP	100 ± 0	78 ± 6.5
3	Reactive Blue 21	control[c]	23 ± 4.1	13 ± 1.6
		*Tfu*DyP	25 ± 4.1	88 ± 3.3
		*Svi*DyP	55 ± 1.6	24 ± 2.4
4	Reactive Red 195	control[c]	0 ± 0	2 ± 0.8
		*Tfu*DyP	69 ± 3.3	6 ± 0
		*Svi*DyP	100 ± 0	68 ± 5.7
5	Reactive Yellow 15	control[c]	0 ± 0	14 ± 0.8
		*Tfu*DyP	37 ± 3.3	20 ± 0.8
		*Svi*DyP	39 ± 0.8	50 ± 4.1
6	Reactive Yellow 42	control[c]	0 ± 1	18 ± 0.8
		*Tfu*DyP	6 ± 1.6	36 ± 2.5
		*Svi*DyP	0 ± 0	51 ± 3.3
7	Reactive Red 120	control[c]	0 ± 0	0 ± 0
		*Tfu*DyP	3 ± 0	24 ± 0.8
		*Svi*DyP	59 ± 0.4	83 ± 2.4
8	Reactive Blue 182	control[c]	0 ± 0	0 ± 0
		*Tfu*DyP	31 ± 0.2	38 ± 6.5
		*Svi*DyP	38 ± 2.4	86 ± 3.2
9	Reactive Black 5	control[c]	–	0 ± 0
		*Tfu*DyP	–	0 ± 0
		*Svi*DyP	–	66 ± 0.8
10	Reactive Blue 171	control[c]	–	21 ± 0.8
		*Tfu*DyP	–	33 ± 2.4
		*Svi*DyP	–	64 ± 3.3
11	Reactive Yellow 84	control[c]	–	0 ± 0
		*Tfu*DyP	–	49 ± 4.9
		*Svi*DyP	–	58 ± 6.5

Table S1. Results for degradation reactions applying *Svi*DyP and *Tfu*DyP in pH 3 (cont.)

entry	Dye	Enzyme	E in 30 min (pH 3)[a,b] / %	E in 12 h (pH 3)[a,b] / %
		control[c]	–	2 ± 0
12	Reactive Yellow 176	*Tfu*DyP	–	9 ± 0.8
		*Svi*DyP	–	57 ± 5.7

[a]Dye (50 µmol L^{-1}), H_2O_2 (100 µmol L^{-1}), enzyme (300 nmol L^{-1}), phosphate buffer pH 3 (total volume = 1 mL); [b]$E(\%) = [(Abs_{initial} - Abs_{final})/Abs_{initial}] \times 100$, quoted results are the average based on the triplicate results and the standard deviation are shown; [c]the control reaction is the reaction without enzyme. E: efficiency of degradation; *Tfu*Dyp and *Svi*DyP: DyP peroxidases isolated from *Thermobifida fusca* (TfuDyp) and *Saccharomonospora viridis*, respectively.

Table S2. Results for degradation reactions applying *Svi*DyP and *Tfu*DyP in pH 4, 5 and 7

entry	Dye	Enzyme	E in 30 min (pH 4)[a,b] / %	E in 30 min (pH 5)[a,b] / %	E in 30 min (pH 7)[a,b] / %
1	Reactive Green 19	control[c]	0 ± 0	0 ± 0	0 ± 0
		*Tfu*DyP	0 ± 0	4 ± 0	10 ± 0
		*Svi*DyP	0 ± 0	17 ± 2.4	12 ± 1.6
2	Reactive Blue 198	control[c]	35 ± 3.3	8 ± 4.9	7 ± 1
		*Tfu*DyP	50 ± 4.1	36 ± 4.9	52 ± 4.9
		*Svi*DyP	42 ± 0.8	56 ± 4.9	42 ± 0.8
3	Reactive Blue 21	control[c]	20 ± 1.6	30 ± 3.3	0 ± 0
		*Tfu*DyP	20 ± 3.3	34 ± 0.8	29 ± 4.1
		*Svi*DyP	0 ± 0	37 ± 4	19 ± 0.8
4	Reactive Red 195	control[c]	8 ± 0.8	0 ± 0	0 ± 0
		*Tfu*DyP	8 ± 1.6	3 ± 0	10 ± 0.8
		*Svi*DyP	12 ± 0	4 ± 0.8	9 ± 0
5	Reactive Yellow 15	control[c]	10 ± 1.6	0 ± 0	0 ± 0
		*Tfu*DyP	11 ± 0.8	0 ± 0	17 ± 1.6
		*Svi*DyP	12 ± 2.4	0 ± 0	42 ± 1.6
6	Reactive Yellow 42	control[c]	0 ± 0	4 ± 1.6	0 ± 0
		*Tfu*DyP	0 ± 0	16 ± 1.6	10 ± 4.1
		*Svi*DyP	0 ± 0	12 ± 2.4	14 ± 3.3
7	Reactive Red 120	control[c]	0 ± 0	0 ± 0	5 ± 0.8
		*Tfu*DyP	24 ± 4.1	4 ± 0	9 ± 1.6
		*Svi*DyP	30 ± 4.9	0 ± 0	9 ± 2.4
8	Reactive Blue 182	control[c]	8 ± 0.8	21 ± 4.9	0 ± 0
		*Tfu*DyP	11 ± 1.6	66 ± 4.1	12 ± 1.6
		*Svi*DyP	30 ± 1.6	72 ± 4.9	26 ± 0.8

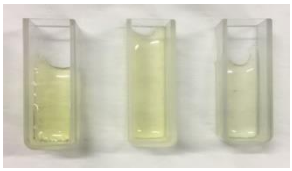
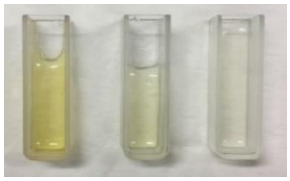
[a]Dye (50 µmol L^{-1}), H_2O_2 (100 µmol L^{-1}), enzyme (300 nmol L^{-1}), corresponding buffer (total volume = 1 mL); [b]E(%) = $[(Abs_{initial} - Abs_{final})/Abs_{initial}] \times 100$, quoted results are the average based on the triplicate results and the standard deviation are shown; [c]the control reaction is the reaction without enzyme. E: efficiency of degradation; *Tfu*Dyp and *Svi*DyP: DyP peroxidases isolated from *Thermobifida fusca* (TfuDyp) and *Saccharomonospora viridis*, respectively.

Table S3. Activity of *Tfu*DyP and *Svi*DyP in the presence of different dyes[a]

entry	Dye	Enzyme	λmax at pH 3 / nm	k_{obs} pH 3 / s^{-1}	λmax at pH 4 / nm	k_{obs} pH 4 / s^{-1}	λmax at pH 5 / nm	k_{obs} pH 5 / s^{-1}	λmax at pH 7 / nm	k_{obs} pH 7 / s^{-1}
1	Reactive Green 19	*Tfu*DyP	629	9.4×10^{-3}	629	5.3×10^{-4}	630	1.5×10^{-3}	628	6.6×10^{-3}
		*Svi*DyP		7.6×10^{-3}		6.0×10^{-4}		3.0×10^{-3}		2.6×10^{-3}
2	Reactive Blue 198	*Tfu*DyP	773	3.3×10^{-3}	635	5.3×10^{-3}	628	2.2×10^{-4}	628	8.1×10^{-4}
		*Svi*DyP		7.0×10^{-4}		4.0×10^{-3}		1.9×10^{-2}		4.0×10^{-3}
3	Reactive Blue 21	*Tfu*DyP	627	2.6×10^{-3}	626	6.0×10^{-4}	627	6.9×10^{-3}	620	3.7×10^{-3}
		*Svi*DyP		4.4×10^{-3}		7.2×10^{-3}		8.5×10^{-3}		3.7×10^{-3}
4	Reactive Red 195	*Tfu*DyP	545	5.8×10^{-3}	540	5.0×10^{-4}	541	7.3×10^{-3}	543	3.5×10^{-3}
		*Svi*DyP		5.1×10^{-3}		4.1×10^{-4}		2.8×10^{-4}		1.1×10^{-3}
5	Reactive Yellow 15	*Tfu*DyP	414	2.3×10^{-2}	414	5.4×10^{-5}	416	3.3×10^{-4}	417	2.2×10^{-4}
		*Svi*DyP		1.5×10^{-3}		1.1×10^{-3}		4.1×10^{-3}		3.7×10^{-3}
6	Reactive Yellow 42	*Tfu*DyP	414	5.6×10^{-3}	416	9.5×10^{-3}	416	4.1×10^{-4}	414	3.9×10^{-3}
		*Svi*DyP		1.2×10^{-3}		2.1×10^{-3}		1.2×10^{-2}		7.2×10^{-4}
7	Reactive Red 120	*Tfu*DyP	535	2.7×10^{-3}	514	1.3×10^{-2}	511	9.9×10^{-3}	512	5.8×10^{-3}
		*Svi*DyP		3.9×10^{-2}		1.3×10^{-3}		3.3×10^{-4}		1.4×10^{-3}
8	Reactive Blue 182	*Tfu*DyP	620	9.8×10^{-3}	620	6.8×10^{-4}	620	5.0×10^{-3}	616	2.6×10^{-3}
		*Svi*DyP		1.0×10^{-2}		9.2×10^{-3}		3.2×10^{-2}		4.4×10^{-3}
9	Reactive Black 5	*Tfu*DyP	596	2.2×10^{-3}	600	2.4×10^{-3}	598	1.6×10^{-4}	600	1.6×10^{-3}
		*Svi*DyP		9.4×10^{-4}		2.5×10^{-3}		2.6×10^{-3}		3.2×10^{-3}
10	Reactive Blue 171	*Tfu*DyP	605	1.0×10^{-3}	605	3.6×10^{-3}	597	8.5×10^{-5}	621	1.4×10^{-4}
		*Svi*DyP		5.9×10^{-4}		1.3×10^{-3}		2.5×10^{-3}		5.1×10^{-4}
11	Reactive Yellow 84	*Tfu*DyP	406	1.3×10^{-3}	410	1.5×10^{-2}	415	5.8×10^{-3}	414	1.6×10^{-3}
		*Svi*DyP		4.5×10^{-4}		2.4×10^{-3}		6.5×10^{-3}		1.0×10^{-3}
12	Reactive Yellow 176	*Tfu*DyP	410	1.8×10^{-2}	430	2.7×10^{-3}	403	1.8×10^{-3}	413	6.1×10^{-4}
		*Svi*DyP		3.1×10^{-3}		8.1×10^{-3}		3.0×10^{-3}		1.1×10^{-3}

[a]Dye (50 μmol L^{-1}), H_2O_2 (100 μmol L^{-1}), enzyme (300 nmol L^{-1}), corresponding buffer (total volume = 1 mL). k_{obs}: initial activity; λmax: maximum wavelength; *Tfu*Dyp and *Svi*DyP: DyP peroxidases isolated from *Thermobifida fusca* (TfuDyp) and *Saccharomonospora viridis*, respectively.

Table S4. Results for the 12 h degradation reactions of different dyes applying *Svi*DyP and *Tfu*DyP and carried out in pH 3

entry	Dye	Enzyme	E (pH 3)[a,b] / %	Initial solution of dye[c]	Solution of the dye after 12 h[d]
1		control[c]	13 ± 1.6 (23)		
2	RB21	*Tfu*DyP	88 ± 3.3 (23)		
3		*Svi*DyP	24 ± 2.4 (23)		
4		control[c]	14 ± 0.8 (5)		
5	RY15	*Tfu*DyP	20 ± 0.8 (5)		
6		*Svi*DyP	50 ± 4.1 (5)		
7		control[c]	18 ± 0.8 (11)		
8	RY42	*Tfu*DyP	36 ± 2.5 (11)		
9		*Svi*DyP	51 ± 3.3 (11)		
10		control[c]	21 ± 0.8 (30)		
11	RB171	*Tfu*DyP	33 ± 2.4 (30)		
12		*Svi*DyP	64 ± 3.3 (30)		
13		control[c]	0 ± 0 (15)		
14	RY84	*Tfu*DyP	49 ± 4.9 (15)		
15		*Svi*DyP	58 ± 6.5 (15)		
16		control[c]	2 ± 0 (7)		
17	RY176	*Tfu*DyP	9 ± 0.8 (7)		
18		*Svi*DyP	57 ± 5.7 (7)		

[a]Dye (50 μmol L^{-1}), H_2O_2 (100 μmol L^{-1}), enzyme (300 nmol L^{-1}), phosphate buffer pH (total volume = 1 mL). At 4 h intervals, and over a total reaction time of 12 h, additional enzyme (0.05 nmol L^{-1}) and H_2O_2 (0.2 mmol L^{-1}) were added to the reaction mixture. Percentage of E with H_2O_2 but without enzyme are given between parenthesis; [b]quoted results are the average based on the triplicate results and the standard deviation are shown; [c]dye (50 μmol L^{-1}), 50 mmol L^{-1} phosphate buffer pH 3 (total volume = 1 mL); [d]the pictures show first the control reaction without enzyme; second, the reaction applying *Tfu*DyP, and third, the reaction applying *Svi*DyP. E: efficiency of degradation; RB21: Reactive Blue 21; RY15: Reactive Yellow 15; RY42: Reactive Yellow 42; RB171: Reactive Blue 171; RY84: Reactive Yellow 84; RY176: Reactive Yellow 176; *Tfu*Dyp and *Svi*DyP: DyP peroxidases isolated from *Thermobifida fusca* (TfuDyp) and *Saccharomonospora viridis*, respectively.

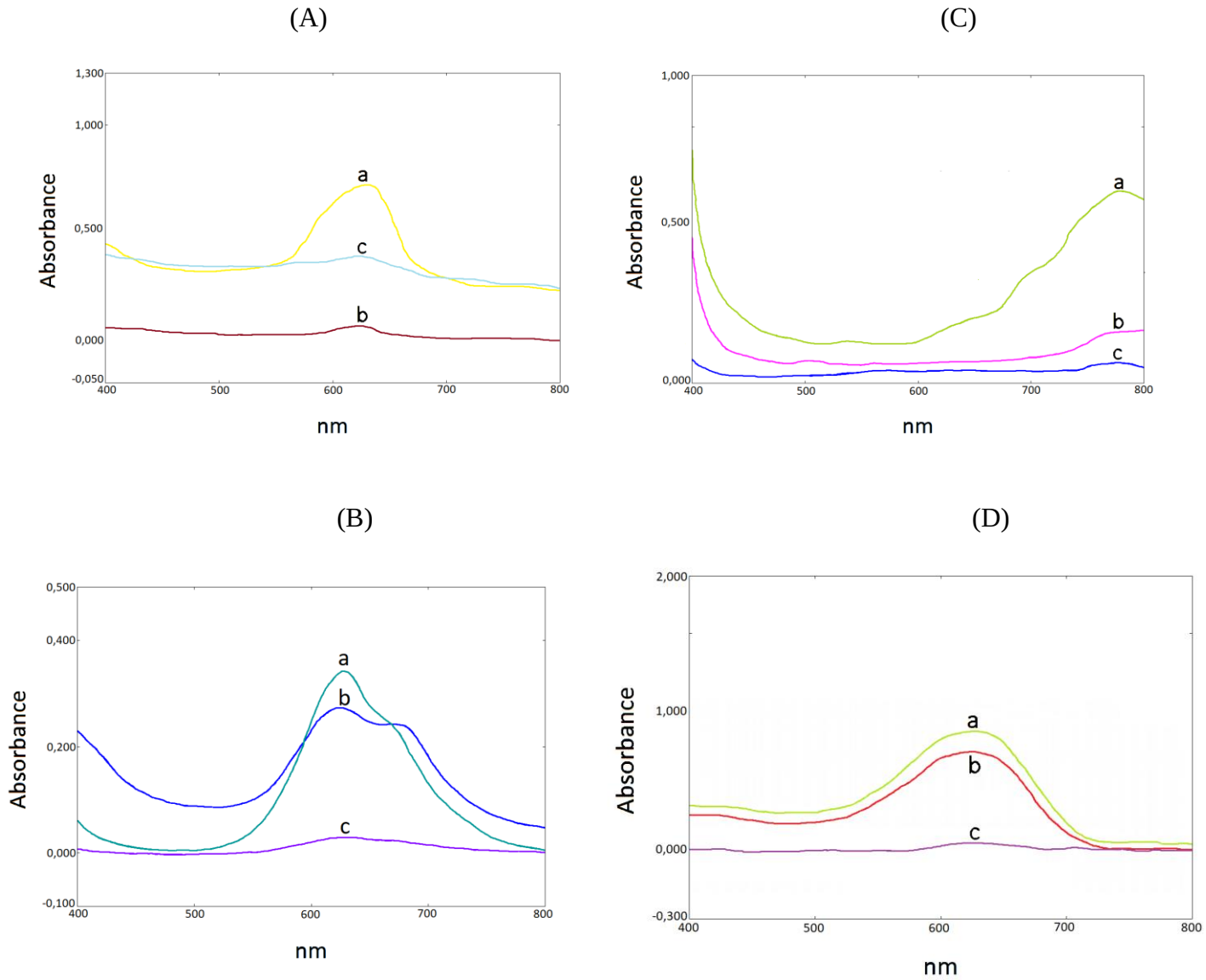


Figure S1. Representative UV-Vis spectra (absorbance × l (nm)) containing degradation results applying pH 3 in 12 h. (A) Reactive Blue 21, (B) Reactive Blue 182, (C) Reactive Blue 198 and (D) Reactive Green 19. (a) Control reaction (without enzyme); (b) reaction applying *Tfu*DyP; (c) reaction applying *Svi*DyP.

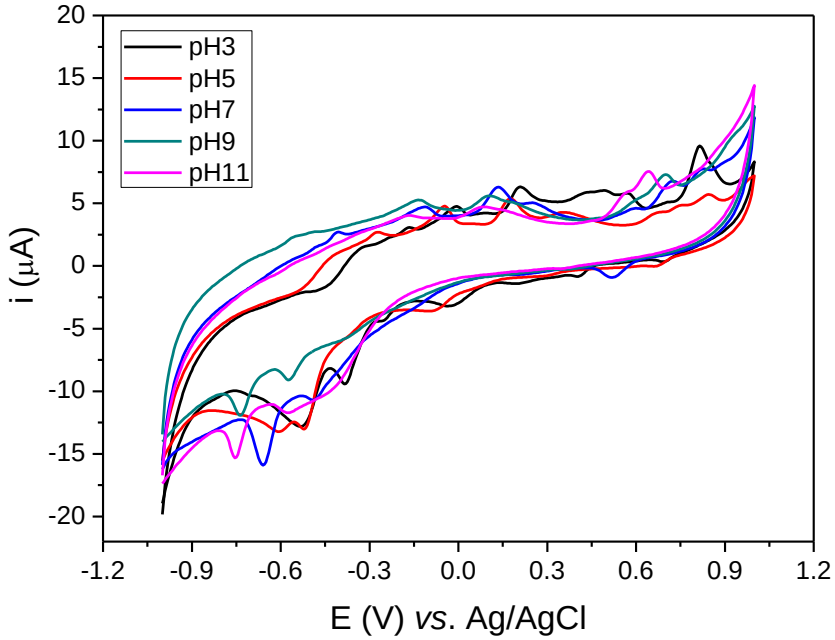


Figure S2. Cyclic voltammogram of Reactive Blue 182 (RB182) in Britton-Robinson buffer solution (0.12 mol L^{-1}), pH ranging from 3.0 to 11.0, scan realized from −1.0 to +1.0 V back to −1.0 V *vs*. Ag/AgCl, at scan rate of 0.05 V s^{-1}.

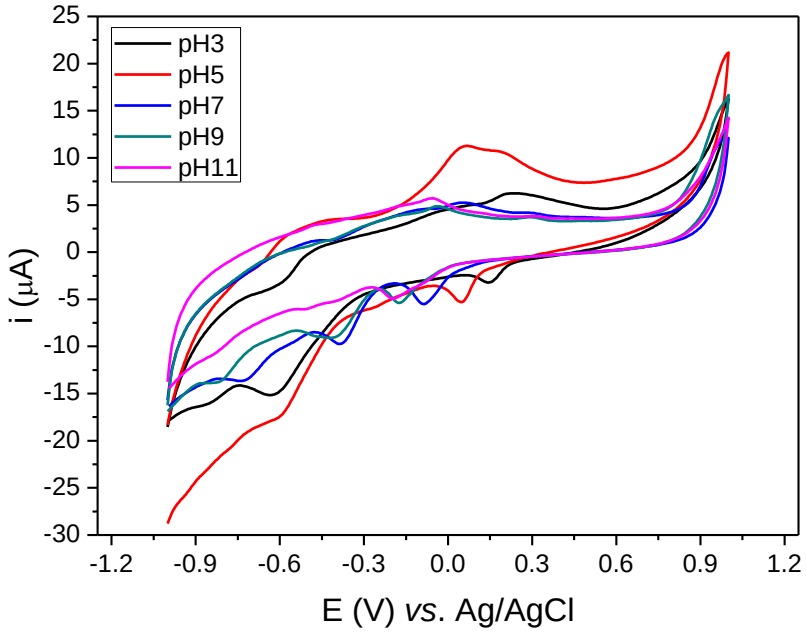


Figure S3. Cyclic voltammogram of Reactive Red 195 (RR195) in Britton-Robinson buffer solution (0.12 mol L^{-1}), pH ranging from 3.0 to 11.0, scan realized from −1.0 to +1.0 V back to −1.0 V *vs*. Ag/AgCl, at scan rate of 0.05 V s^{-1}.

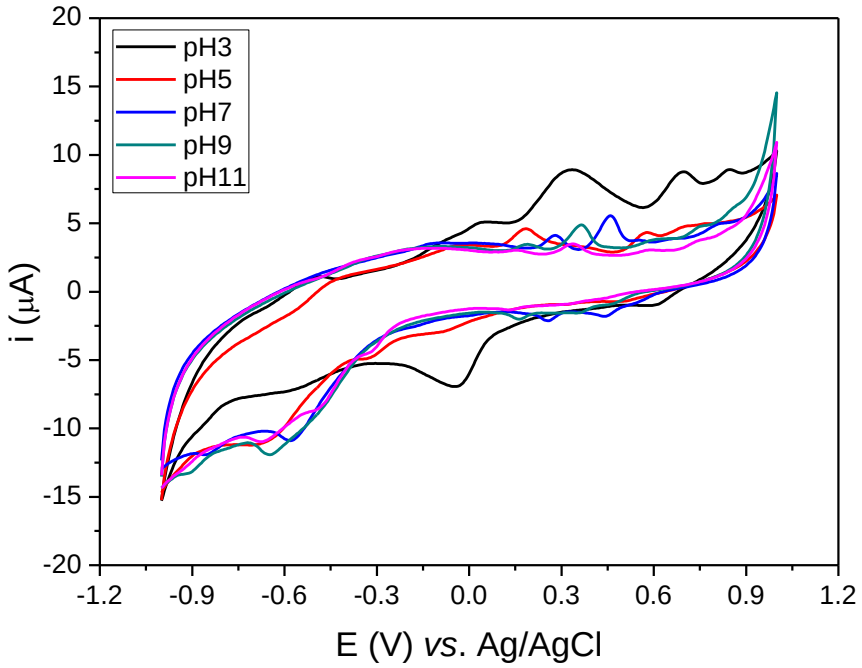


Figure S4. Cyclic voltammogram of Reactive Green 19 (RG19) in Britton-Robinson buffer solution (0.12 mol L^{-1}), pH ranging from 3.0 to 11.0, scan realized from –1.0 to +1.0 V back to –1.0 V *vs*. Ag/AgCl, at scan rate of 0.05 V s^{-1}.

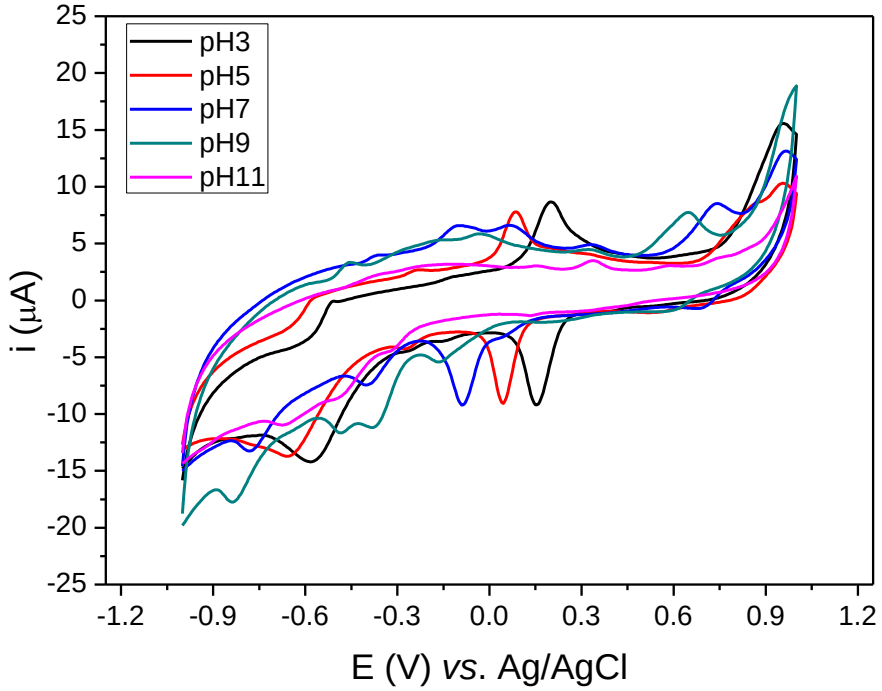


Figure S5. Cyclic voltammogram of Reactive Red 120 (RR120) in Britton-Robinson buffer solution (0.12 mol L^{-1}), pH ranging from 3.0 to 11.0, scan realized from –1.0 to +1.0 V back to –1.0 V *vs*. Ag/AgCl, at scan rate of 0.05 V s^{-1}.

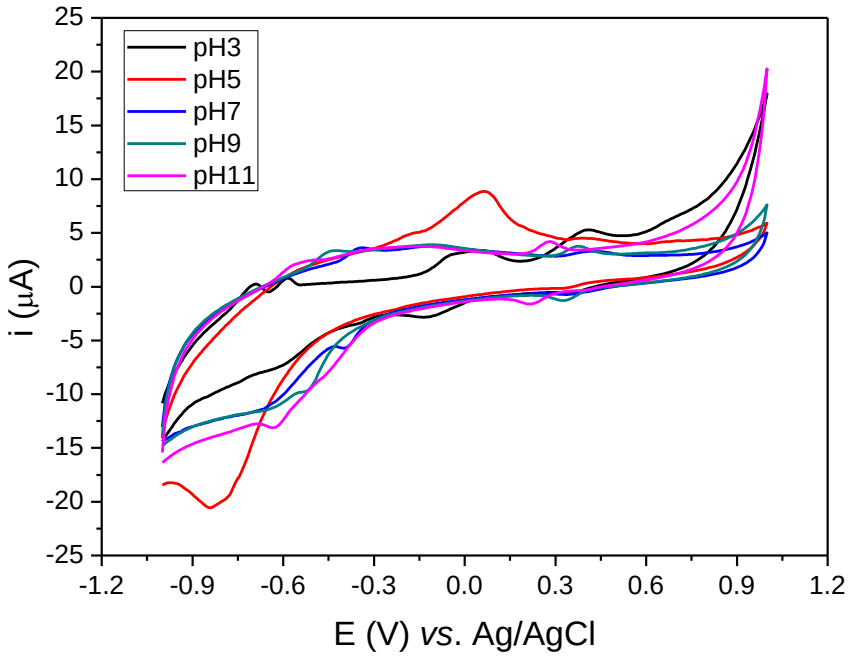


Figure S6. Cyclic voltammogram of Reactive Blue 198 (RB198) in Britton-Robinson buffer solution (0.12 mol L^{-1}), pH ranging from 3.0 to 11.0, scan realized from –1.0 to +1.0 V back to –1.0 V *vs*. Ag/AgCl, at scan rate of 0.05 V s^{-1}.

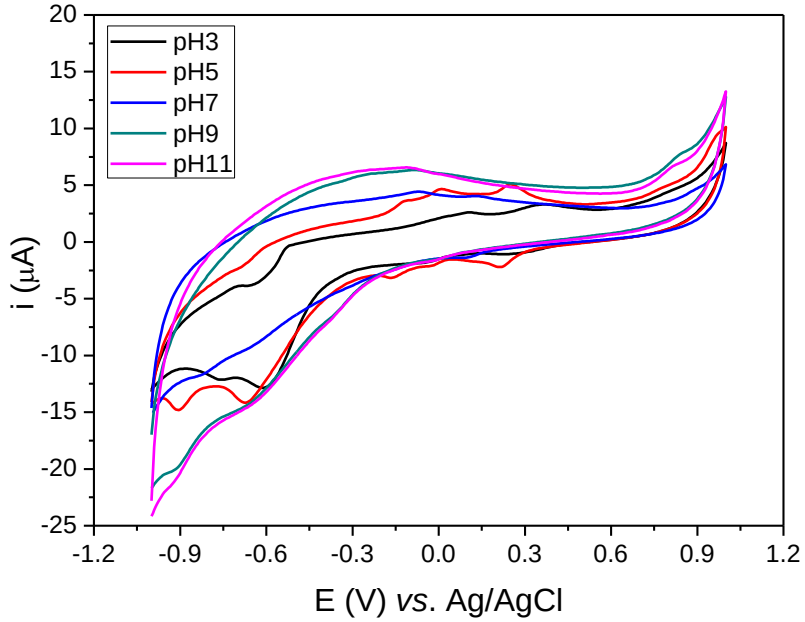


Figure S7. Cyclic voltammogram of Reactive Yellow 42 (RY42) in Britton-Robinson buffer solution (0.12 mol L^{-1}), pH ranging from 3.0 to 11.0, scan realized from –1.0 to +1.0 V back to –1.0 V *vs*. Ag/AgCl, at scan rate of 0.05 V s^{-1}.

Reactive Blue 21 (RB 21) was not detected by cyclic voltammetry, so it was evaluated by square wave voltammetry, potential range from –1.7 to +1.7 V, frequency of 75 Hz, amplitude of 0.05 V and potential increment of 0.005 V.

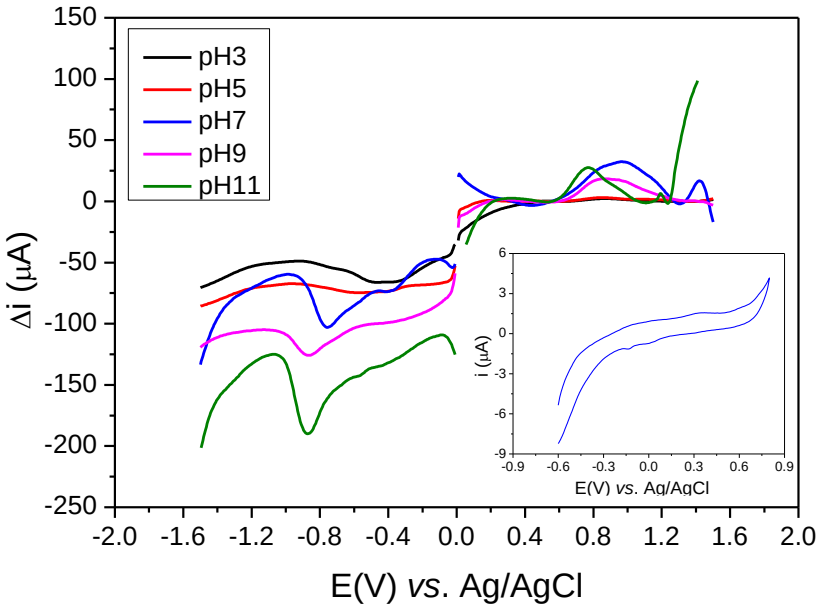


Figure S8. Square-wave voltammogram of Reactive Blue 21 (RB21) in Britton-Robinson buffer solution (0.12 mol L^{-1}), pH ranging from 3.0 to 11.0, scan realized from –1.7 to +1.7 V, frequency of 75 Hz, amplitude of 0.05 V and potential increment of 0.005 V. Inset: cyclic voltammogram at pH 3.0.

*Tfu*DyP and *Svi*DyP cyclic voltammograms, performed in BRBS (0.12 mol L^{-1}), in pH 3.0 and 7.0. Potential window evaluated was from –0.5 to +0.5 V, at scan rate of 0.1 V s^{-1}.

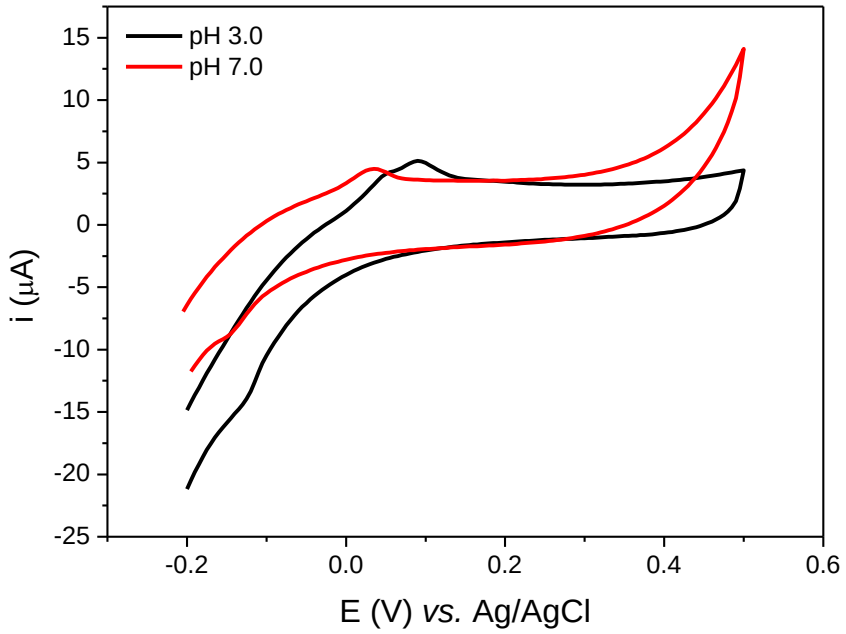


Figure S9. Cyclic voltammogram of *Tfu*DyP in Britton-Robinson Buffer solution (0.12 mol L^{-1}), pH of 3.0 and 7.0 at scan rate of 0.05 V s^{-1}.

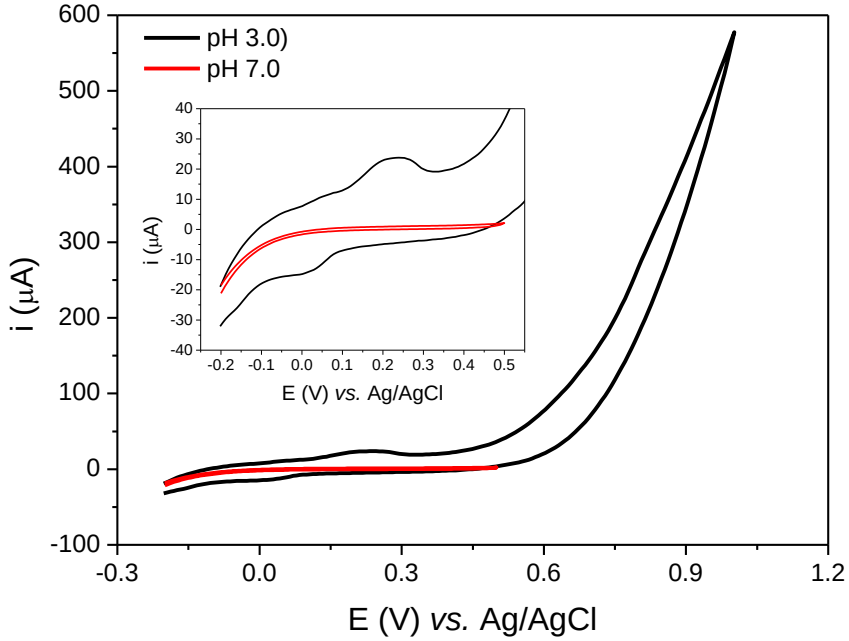
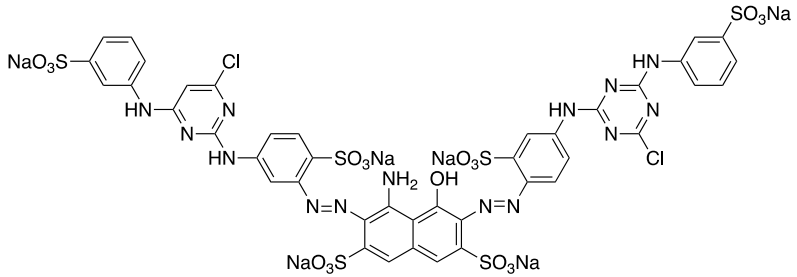
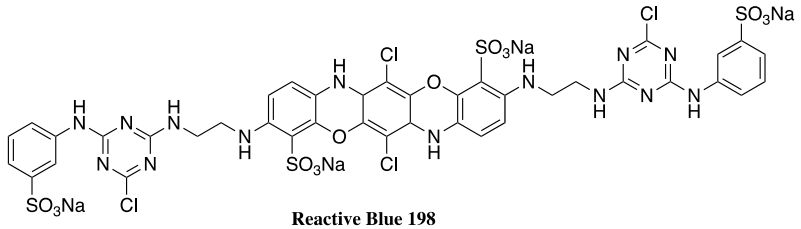


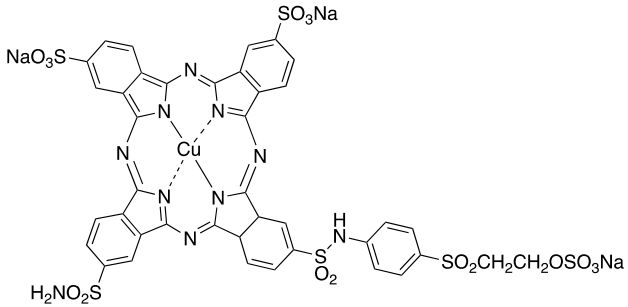
Figure S10. Cyclic voltammogram of *Svi*DyP in Britton-Robinson Buffer solution (0.12 mol L^{-1}), pH of 3.0 and 7.0 at scan rate of 0.05 V s^{-1}. Inset: zoom in the redox process.



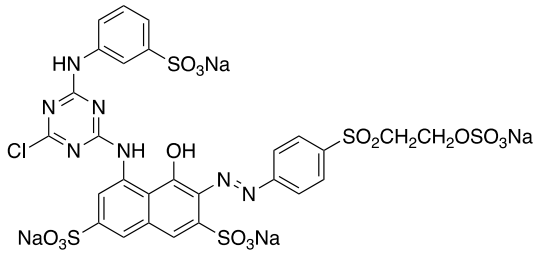
Reactive Green 19
(RG 19)



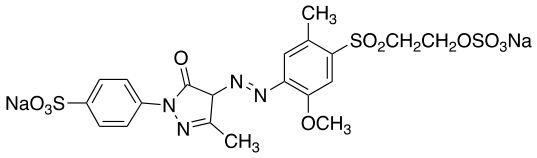
Reactive Blue 198
(RB 198)



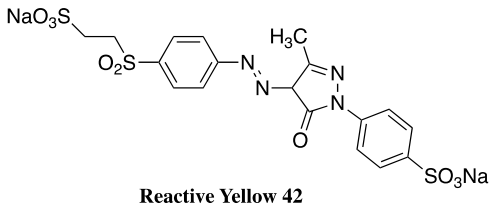
Reactive Blue 21
(RB 21)



Reactive Red 195
(RR 195)

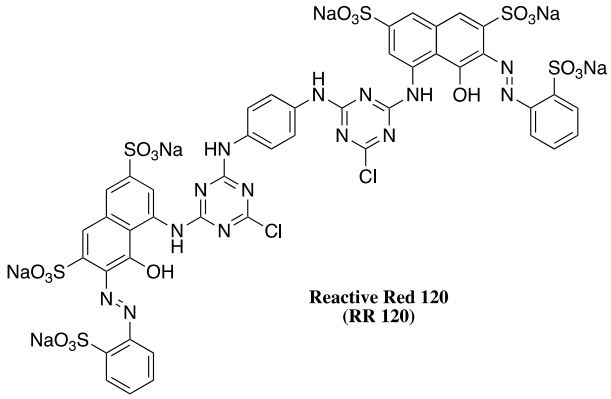


Reactive Yellow 15 195
(RY 15)

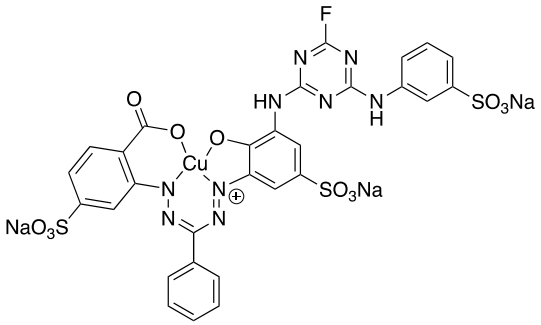


Reactive Yellow 42
(RY 42)

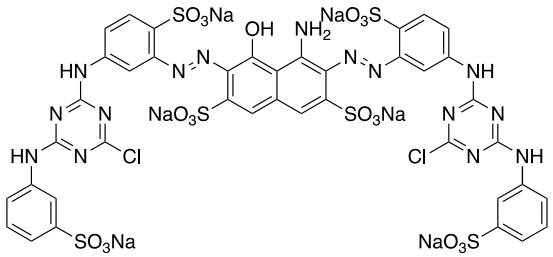
Figure S11. Chemical structure of the selected dyes.



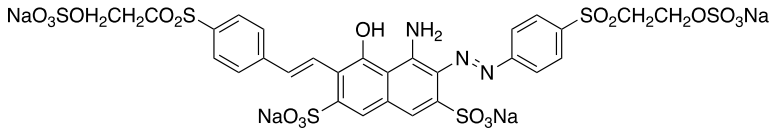
Reactive Red 120
(RR 120)



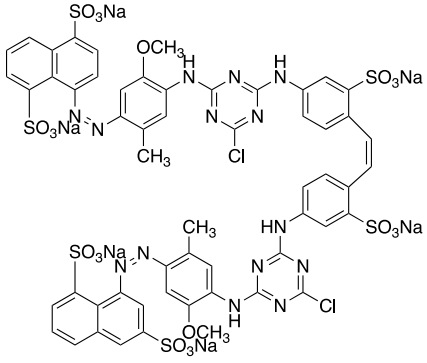
Reactive Blue 182
(RB 182)



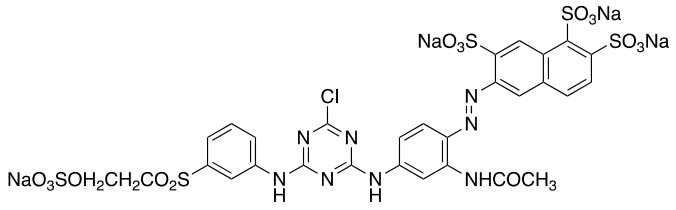
Reactive Blue 171
(RB 171)



Reactive Black 5
(RB 5)



Reactive Yellow 84
(RY 84)



Reactive Yellow 176
(RY 176)

Figure S12. Chemical structure of the selected dyes.