

## Supplemental Information

### Improved HPLC Conditions to Determine Eumelanin and Pheomelanin Contents in Biological Samples Using an Ion Pair Reagent

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**Table S1.** Comparison of levels of melanin markers in synthetic melanins between the improved HPLC method and the original HPLC method (n = 12)

Type of Melanin	AHPO ( $\mu\text{g}/\text{mg}$ ) <sup>a</sup>				
	PTCA	PDCA	PTeCA	TTCA	TDCA
Dopa-M, native	5.37 / 4.96	7.17 / 7.27	1.84 / 1.25	0.00 / 0.13	0.00 / 0.09
Dopa+Cys (1:0.5)-M, native	5.24 / 4.04	4.22 / 4.27	0.95 / 0.70	5.76 / 6.55	3.55 / 3.80
Dopa+Cys (1:1)-M, native	4.34 / 3.83	1.17 / 1.03	0.24 / 0.17	5.52 / 6.82	2.07 / 2.30
DHI+DHICA (1:0)-M, native	2.38 / 2.32	1.81 / 3.04	4.40 / 3.82	0.00 / 0.00	0.00 / 0.00
DHI+DHICA (1:1)-M, native	41.7 / 39.8	1.35 / 2.08	2.84 / 2.14	0.00 / 0.00	0.00 / 0.00
DHI+DHICA (0:1)-M, native	129 / 123	1.76 / 2.52	0.95 / 1.00	0.00 / 0.00	0.00 / 0.00
Dopa-M, heated	7.50 / 6.89	6.43 / 6.87	2.95 / 2.03	0.00 / 0.31	0.00 / 0.13
Dopa+Cys (1:0.5)-M, heated	2.57 / 2.37	3.14 / 3.27	1.30 / 0.90	10.75 / 12.31	8.79 / 8.78
Dopa+Cys (1:1)-M, heated	2.49 / 2.38	0.90 / 0.97	0.24 / 0.46	11.65 / 13.43	6.54 / 6.88
DHI+DHICA (1:0)-M, heated	2.90 / 2.87	1.30 / 3.56	8.01 / 7.59	0.00 / 0.00	0.00 / 0.00
DHI+DHICA (1:1)-M, heated	8.93 / 8.21	0.93 / 1.95	4.71 / 4.26	0.00 / 0.00	0.00 / 0.00
DHI+DHICA (0:1)-M, heated	12.7 / 11.5	0.73 / 1.49	4.80 / 4.52	0.00 / 0.00	0.00 / 0.00
Averages	18.8 / 17.7 (n = 12)	2.58 / 3.19 (n = 12)	2.77 / 2.40 (n = 12)	5.61 / 6.50 (n = 4)	3.49 / 3.66 (n = 6)

<sup>a</sup> Values are reported for the improved HPLC method / the original HPLC method. For example, 5.37 PTCA is the value with the improved HPLC method while 4.96 is with the original HPLC method.

**Table S2.** Comparison of levels of melanin markers in mouse hair samples between the improved HPLC method and the original HPLC method (n = 10).

Coat color phenotype	AHPO (ng/mg) <sup>a</sup>			
	PTCA	PDCA	PTeCA	TTCA
<i>Black</i>	2828 / 2897	25.2 / 40.7	154 / 160	27.8 / 17.8
<i>Black</i>	3406 / 3397	30.0 / 27.1	194 / 186	27.8 / 25.4
<i>Brown</i>	787 / 812	17.6 / 23.3	34.1 / 29.3	23.2 / 20.4
<i>Brown</i>	877 / 854	22.9 / 16.1	47.5 / 29.3	23.2 / 42.6
<i>Slaty</i>	389 / 399	81.7 / 88.5	41.3 / 53.3	37.1 / 30.7
<i>Slaty</i>	525 / 514	107 / 103	63.2 / 67.3	51.0 / 47.4
<i>Pink-eyed dilution</i>	144 / 150	8.1 / 4.2	12.4 / 8.0	18.5 / 20.4
<i>Pink-eyed dilution</i>	189 / 182	7.5 / 5.2	15.4 / 10.7	13.9 / 18.9
<i>Recessive yellow</i>	129 / 127	30.0 / 32.9	10.3 / 5.3	111 / 102
<i>Recessive yellow</i>	149 / 153	32.7 / 20.8	16.5 / 5.3	125 / 130
Averages	942 / 949	36.3 / 36.2	45.9 / 45.6	58.8 / 55.5

<sup>a</sup> Values are reported for the improved HPLC method / the original HPLC method. For example, 2828 PTCA is the value with the improved method while 2897 is with the original method.

**Table S3.** Comparison of levels of melanin markers in human hair samples between the improved HPLC method and the original HPLC method (n = 12)

Hair color	AHPO (ng/mg) <sup>a</sup>				
	PTCA	PDCA	PTeCA	TTCA	TDCA
Black	178 / 187	18.6 / 21.8	48.1 / 49.1	33.8 / 38.2	17.3 / 21.2
Black	203 / 22.6	20.4 / 22.6	55.5 / 55.8	41.5 / 41.9	18.5 / 20.1
Dark brown	141 / 175	10.8 / 14.7	51.1 / 52.1	27.4 / 40.6	13.6 / 15.5
Dark brown	188 / 210	17.1 / 20.3	62.2 / 61.4	34.0 / 45.0	17.6 / 18.8
Brown	110 / 97.0	8.8 / 7.3	33.3 / 46.9	20.3 / 22.3	12.2 / 10.3
Brown	123 / 128	9.8 / 7.0	43.1 / 46.5	25.0 / 37.8	13.6 / 18.4
Light brown	55.2 / 54.2	3.9 / 4.1	14.8 / 22.3	14.1 / 16.8	9.5 / 8.0
Light brown	50.0 / 50.7	5.0 / 3.5	14.8 / 22.3	17.8 / 19.8	6.8 / 8.0
Blond	8.4 / 9.2	2.0 / 1.2	6.2 / 17.4	8.1 / 10.0	4.1 / 4.6
Blond	6.8 / 6.1	1.5 / 1.2	4.9 / 11.2	6.1 / 5.7	2.7 / 2.3
Red	32.7 / 48.2	6.6 / 4.7	9.9 / 14.9	96.8 / 81.0	21.5 / 19.0
Red	21.9 / 24.2	7.2 / 7.5	7.4 / 14.9	144 / 143	21.6 / 18.9
Averages	93.2 / 99.6	9.3 / 9.7	29.3 / 34.6	39.1 / 41.8	13.3 / 13.8

<sup>a</sup> Values are reported for the improved HPLC method / the original HPLC method. For example, 178 PTCA is the value with the improved method while 187 is with the original method.

**Table S4.** Comparison of levels of melanin markers in human epidermal samples between the improved HPLC method and the original HPLC method (n = 18)

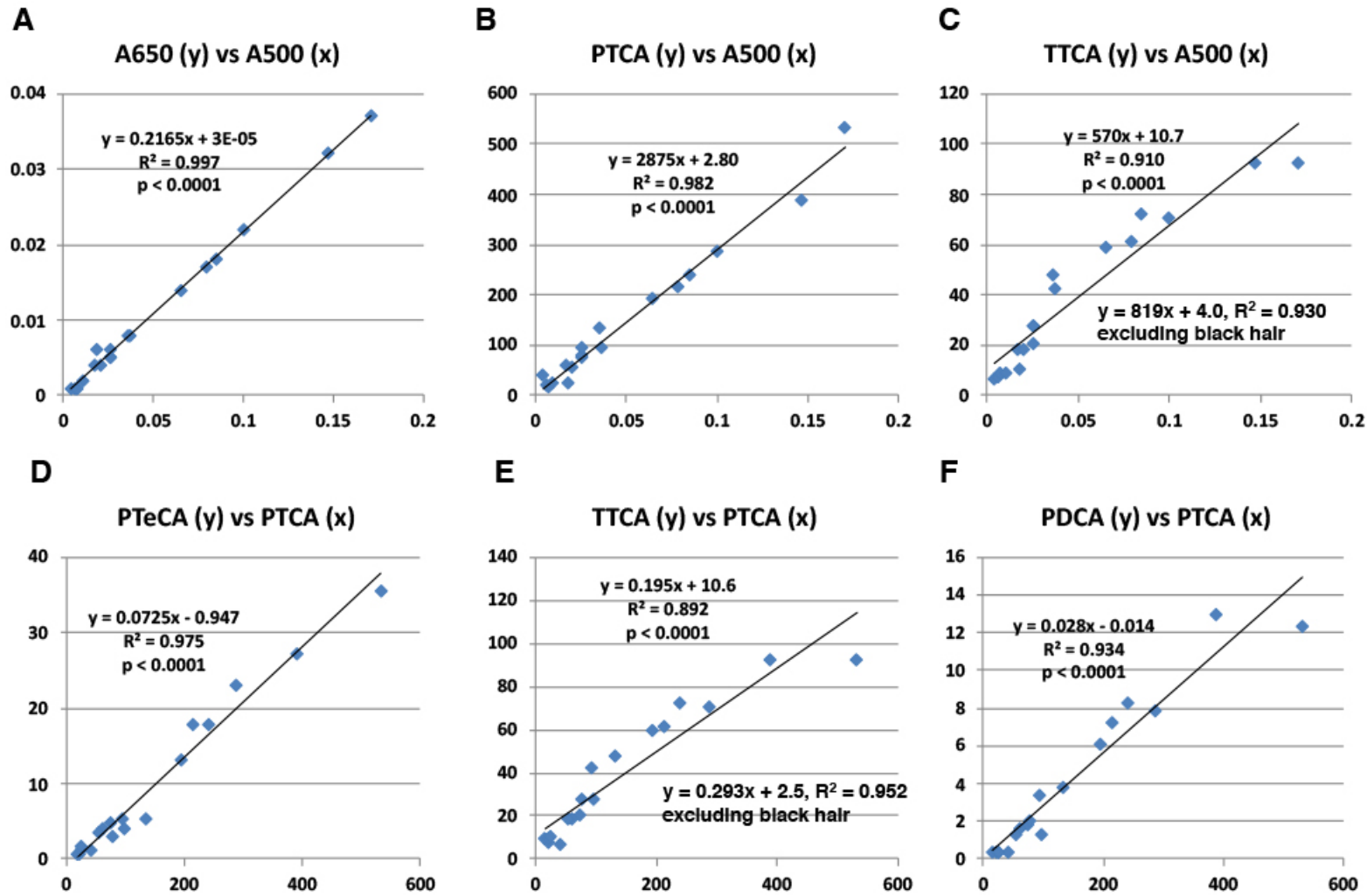
ITA	Skin color typology	Soluen-350 solubilization		AHPO (ng/mg) <sup>a</sup>					HI hydrolysis	
		A500 (/mg)	A650 (/mg)	PTCA	PDCA <sup>b</sup>	PTeCA	TTCA	TDCA	4-AHP	3-AHP
57.92	Very light	0.004	0.001	40.7 / 43,0	0.4 / 3.3	1.2 / 3.8	6.5 / 6.9	3.2 / 4.9	3.3	3.1
55.72	Very light	0.006	0.001	20.9 / 28,2	0.4 / 3.8	0.6 / 5.3	7.4 / 11.3	3.2 / 2.4	4.9	4.3
55.05	Very light	0.007	0.001	15.8 / 18.9	0.4 / 3.8	0.6 / 4.2	9.3 / 8.8	3.2 / 4.9	4.3	2.8
54.08	Light	0.010	0.002	23.9 / 23.9	0.4 / 2.7	1.2 / 3.8	9.3 / 10.1	3.2 / 6.1	4.8	2.9
47.6	Light	0.018	0.006	24.4 / 27.2	0.4 / 5.4	1.8 / 4.5	10.2 / 11.3	3.2 / 9.1	6.6	3.7
41.5	Light	0.026	0.006	74.3 / 86.3	1.8 / 6.0	4.7 / 4.5	20.4 / 22.7	6.3 / 10.3	15.2	6.3
36.03	Intermediate	0.017	0.004	62.1 / 65.1	1.6 / 3.3	4.1 / 4.5	18.5 / 16.6	3.8 / 7.3	14.1	5.8
32.82	Intermediate	0.026	0.005	78.5 / 84.6	2.0 / 7.6	3.0 / 3.8	27.8 / 31.5	5.1 / 9.7	15.7	7.3
29.56	Intermediate	0.020	0.004	55.1 / 59.8	1.3 / 6.5	3.6 / 8.3	18.5 / 15.2	7.6 / 8.5	19.5	7.1
19.21	Tan	0.036	0.008	134 / 140	3.8 / 7.6	5.3 / 6.1	48.1 / 47.9	13.9 / 15.8	38.2	13.6
19.38	Tan	0.037	0.008	93.8 / 95.8	3.4 / 4.4	5.3 / 7.6	42.6 / 41.6	12.7 / 12.2	54.1	18.3
19.12	Tan	0.026	0.005	96.5 / 98.2	1.3 / 5.4	4.1 / 13.6	27.8 / 23.9	10.1 / 9.7	21.2	6.6
-9.96	Brown	0.065	0.014	194 / 197	6.1 / 8.7	13.0 / 16.6	59.2 / 60.9	13.9 / 18.3	43.5	13.6
-0.59	Brown	0.085	0.018	241 / 241	8.3 / 10.9	17.8 / 18.2	72.2 / 74.0	15.2 / 17.9	45.5	14.7
-26.94	Brown	0.079	0.017	215 / 216	7.2 / 8.7	17.8 / 24.2	61.1 / 59.5	20.3 / 20.9	21.8	7.9
-49.5	Dark	0.100	0.022	288 / 284	7.8 / 9.8	23.1 / 31.0	70.3 / 60.9	17.7 / 17.0	26.3	10.0
-50.69	Dark	0.147	0.032	389 / 388	13.0 / 14.1	27.3 / 34.8	92.2 / 82.7	27.8 / 26.2	27.8	9.4
-70.85	Dark	0.171	0.037	533 / 500	12.3 / 13.1	35.6 / 39.0	92.1 / 92.0	30.4 / 29.2	36.4	13.1
Averages		0.0489	0.0106	143/144	3.9/7.0	9.5/13.0	38.5/37.7	12.5/12.8	22.4	8.4

<sup>a</sup> Values by the improved method/original method. For example, 40.7 PTCA is the value with the improved method while 43.0 is with the original method.

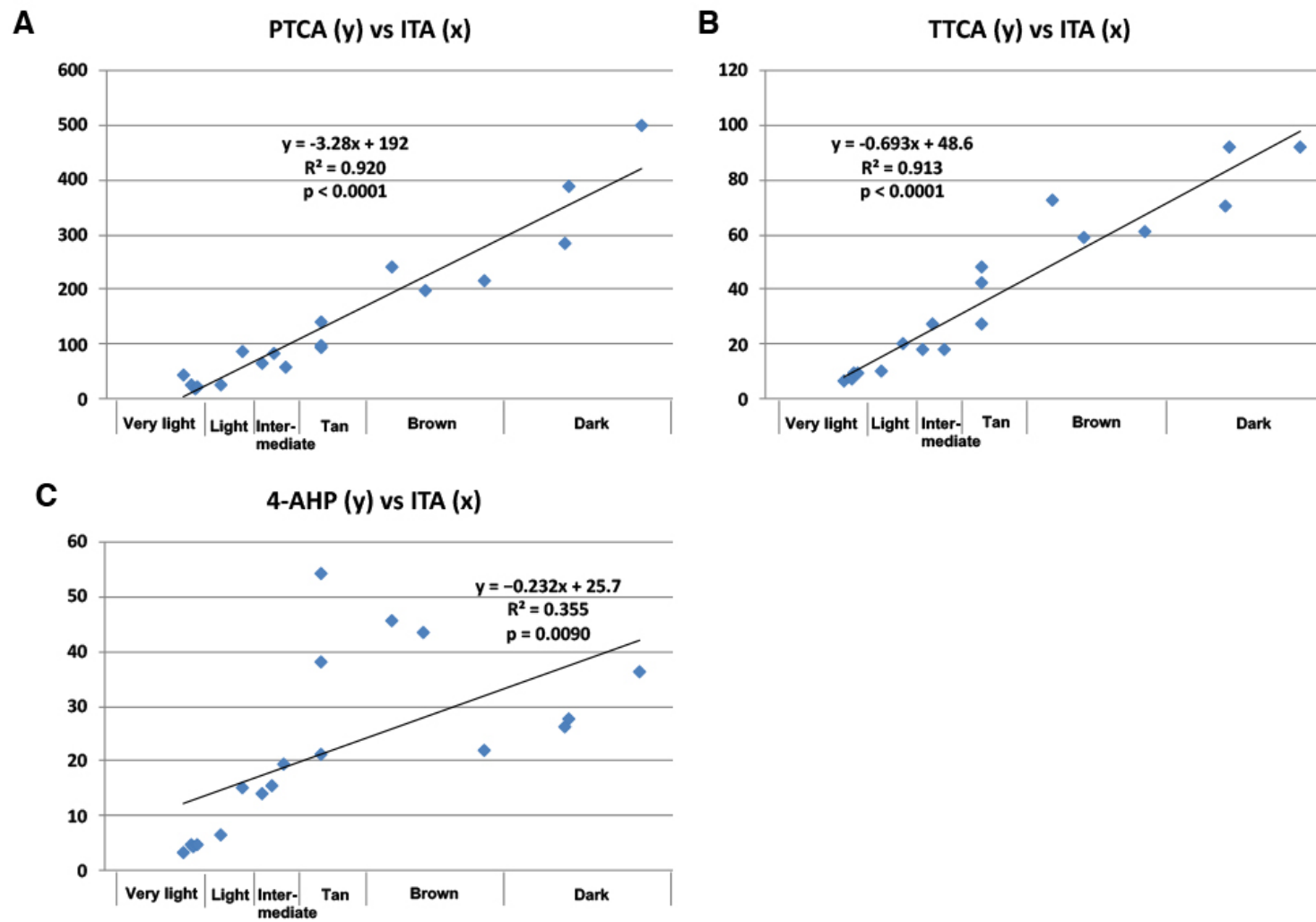
<sup>b</sup> An interfering peak with the original method affected the values.

**Figure S1.** Correlations among various melanin markers in human epidermis. The units on the x- and y-axes are 1/mg for A500 and A650

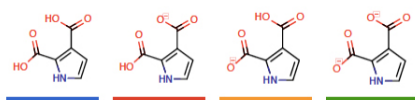
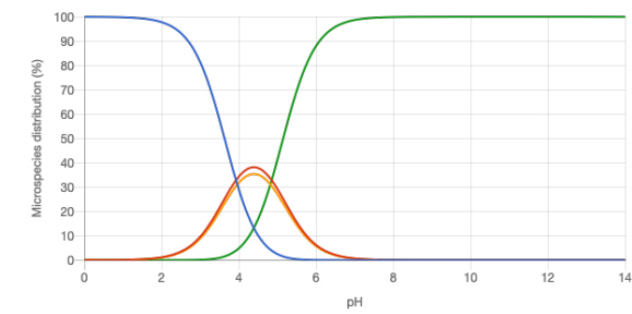
and ng/mg for the other markers.



**Figure S2.** Correlation of melanin markers with ITA. The units for the y-axis is ng/mg.



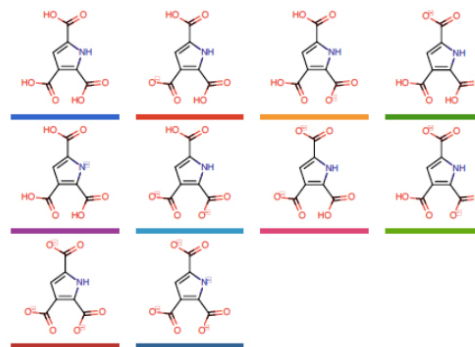
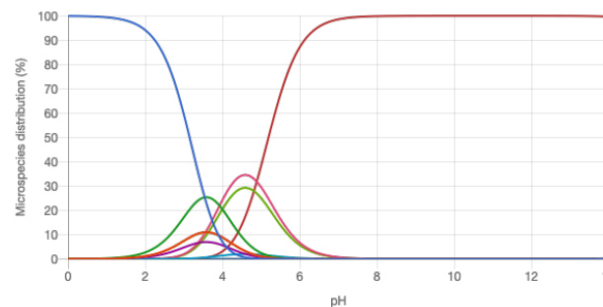
**Figure S3.** Microspecies distribution at various pH and strongest acidic pKa in PDCA, TDCA, PTCA, TTCA, and PTeCA.



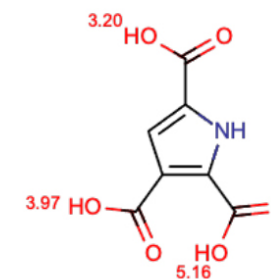
**Strongest acidic pKa: 3.64**



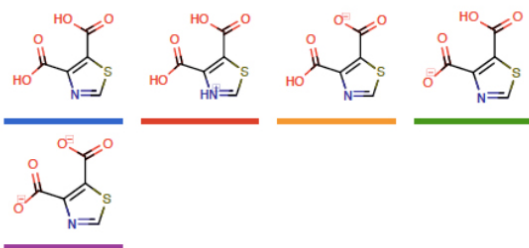
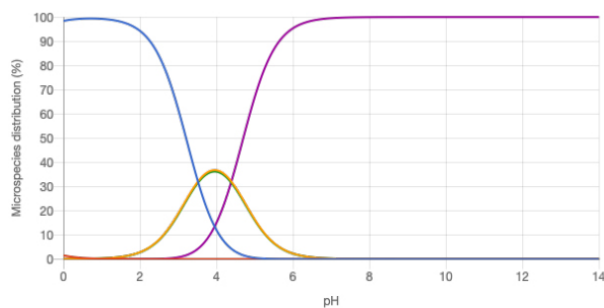
**PDCA**



**Strongest acidic pKa: 3.2**

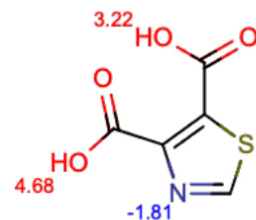


**PTCA**

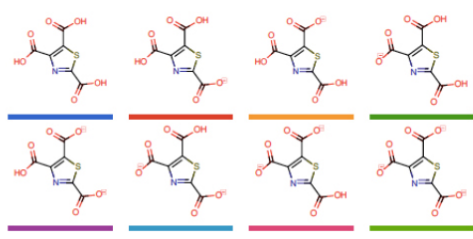
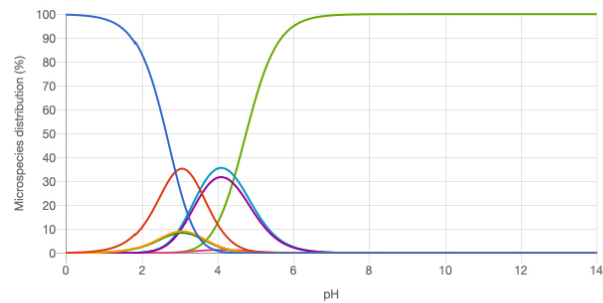


**Strongest acidic pKa: 3.22**

**Strongest basic pKa: -1.81**



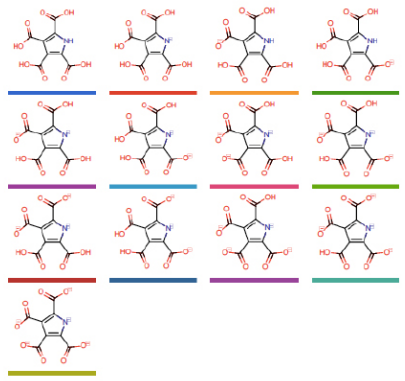
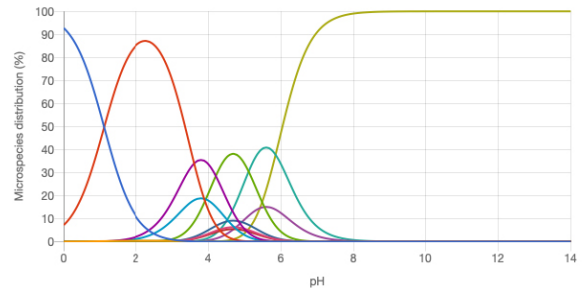
**TDCA**



**Strongest acidic pKa: 2.7**



**TTCA**



**Strongest acidic pKa: 1.11**



**PTeCA**