

# Supplementary Information

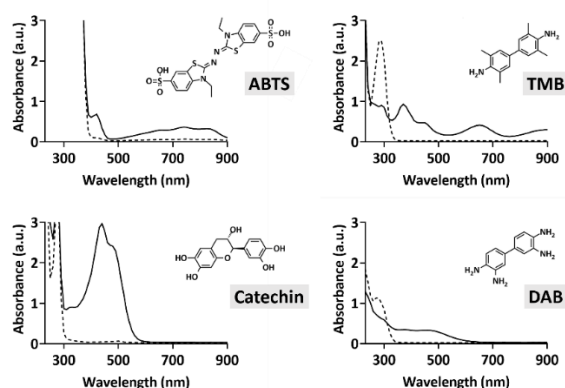
**Site-specific fabrication of a melanin-like pigment through spatially confined progressive assembly on an initiator-loaded template**

*Haejin Jeong, Jisoo Lee, Seunghwi Kim, Haeram Moon, and Seonki Hong\**

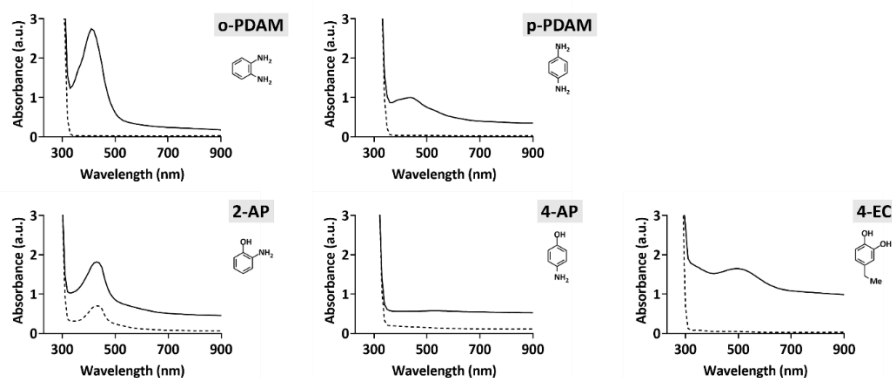
Prof. Dr. S. Hong, H. Jeong, J. Lee, H. Moon, S. Kim

Department of Physics and Chemistry, DGIST, Daegu, 42988, Republic of Korea

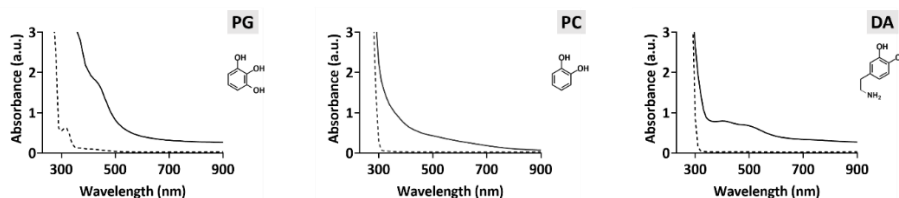
\*Correspondence to: [seonkihong@dgist.ac.kr](mailto:seonkihong@dgist.ac.kr)



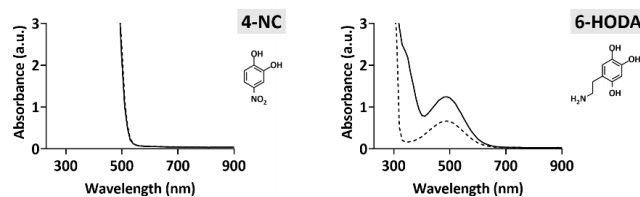
**Supplementary Fig. 1.** UV-vis-NIR spectrum of four class 1 precursors showing defined peaks corresponding to water-soluble chromophores after treatment with HRP/H<sub>2</sub>O<sub>2</sub> (HRP: 10 U/mL, H<sub>2</sub>O<sub>2</sub> 20 mM) in solution for 60 min at 25 °C (dotted line: intrinsic spectrum of precursor before oxidation, solid line: spectrum after HRP/H<sub>2</sub>O<sub>2</sub> oxidation). ABTS: 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), TMB: 3,3',5,5'-Tetramethylbenzidine, DAB: 3,3'-diaminobenzidine, a.u.: arbitrary units.



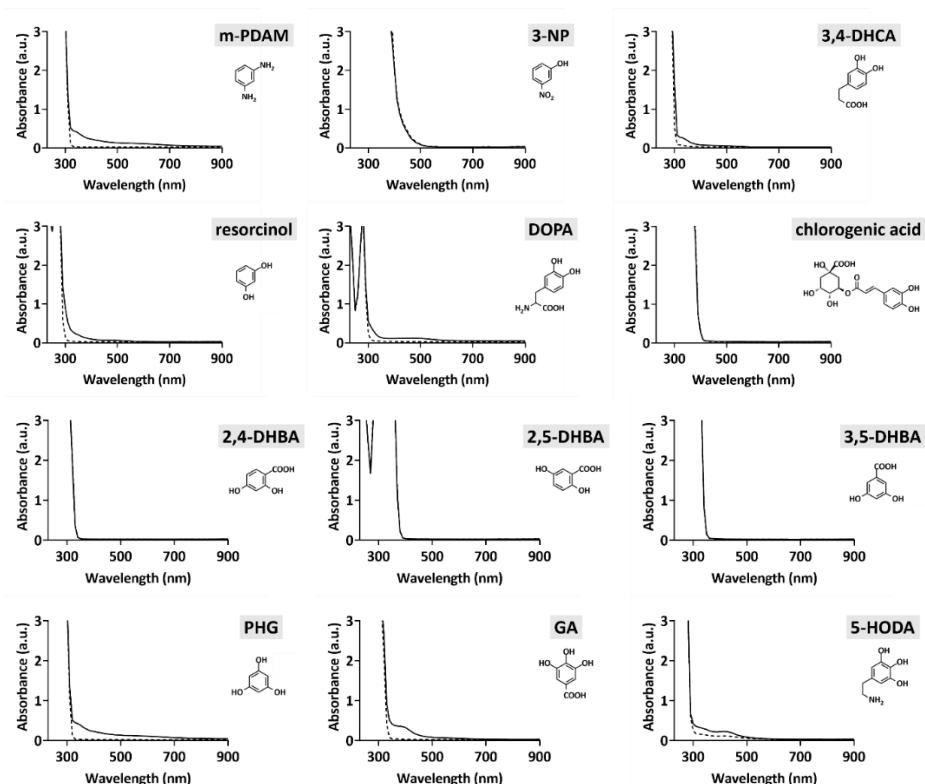
**Supplementary Fig. 2.** UV-vis-NIR spectrum of five class 2 precursors resulting in nano-to-micro-sized particulate formation after treatment with HRP/H<sub>2</sub>O<sub>2</sub> (HRP: 10 U/mL, H<sub>2</sub>O<sub>2</sub> 20 mM) in solution for 60 min at 25 °C (dotted line: intrinsic spectrum of precursor before oxidation, solid line: spectrum after HRP/H<sub>2</sub>O<sub>2</sub> oxidation). PDAM: phenylenediamine, AP: aminophenol, EC: ethylcatechol, a.u.: arbitrary units.



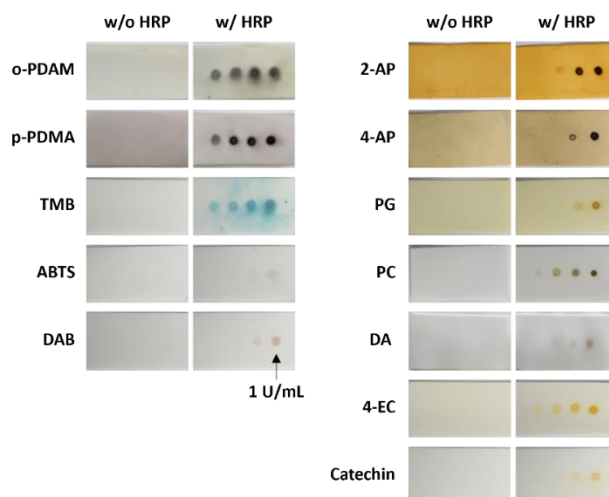
**Supplementary Fig. 3.** UV-vis-NIR spectrum of three class 3 precursors showing melanin-like pigmentation behavior after treatment with HRP/H<sub>2</sub>O<sub>2</sub> (HRP: 10 U/mL, H<sub>2</sub>O<sub>2</sub> 20 mM) in solution for 60 min at 25 °C (dotted line: intrinsic spectrum of precursor before oxidation, solid line: spectrum after HRP/H<sub>2</sub>O<sub>2</sub> oxidation). PG: pyrogallol, PC: pyrocatechol, DA: dopamine, a.u.: arbitrary units.



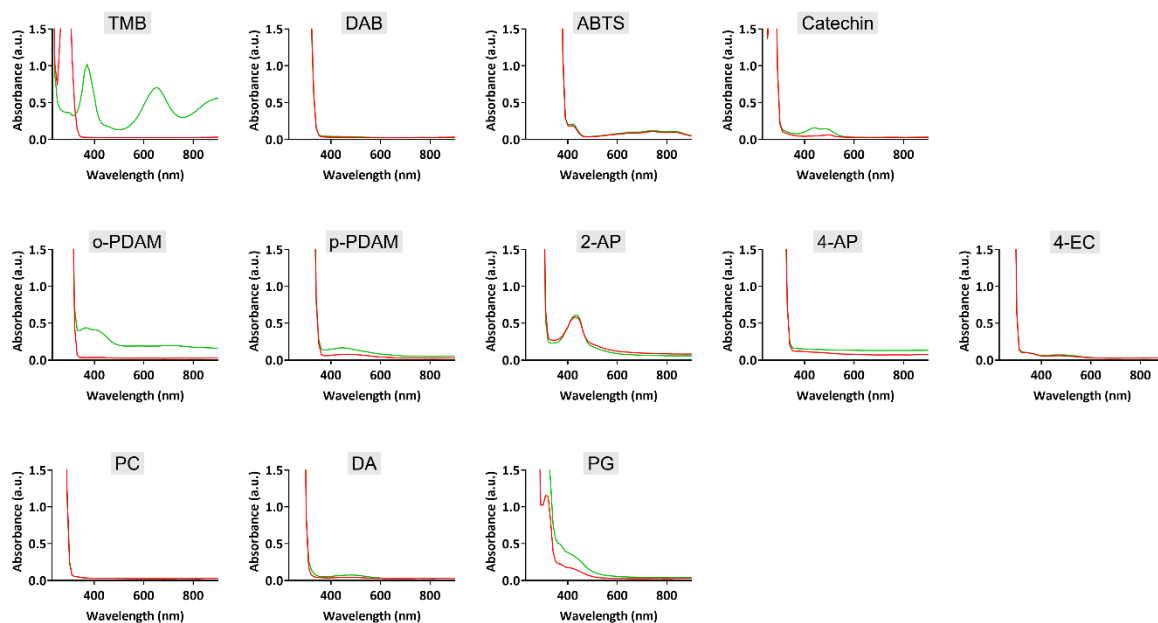
**Supplementary Fig. 4.** UV-vis-NIR spectrum of two class 4 precursors having intrinsic background signals before treatment with HRP/H<sub>2</sub>O<sub>2</sub> (HRP: 10 U/mL, H<sub>2</sub>O<sub>2</sub> 20 mM) in solution for 60 min at 25 °C (dotted line: intrinsic spectrum of precursor before oxidation, solid line: spectrum after HRP/H<sub>2</sub>O<sub>2</sub> oxidation). NC: nitrocatechol, 6-HODA: 6-hydroxydopamine, a.u.: arbitrary units.



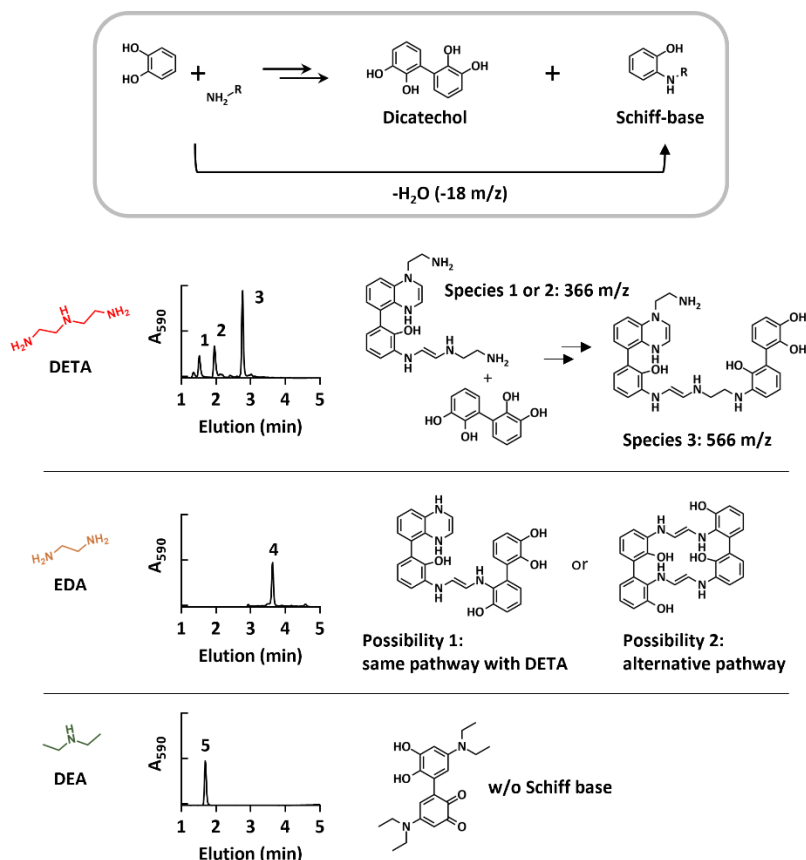
**Supplementary Fig. 5.** UV-vis-NIR spectrum of class 5 precursors having minimum response after treatment with HRP/H<sub>2</sub>O<sub>2</sub> (HRP: 10 U/mL, H<sub>2</sub>O<sub>2</sub> 20 mM) in solution for 60 min at 25 °C (dotted line: intrinsic spectrum of precursor before oxidation, solid line: spectrum after HRP/H<sub>2</sub>O<sub>2</sub> oxidation). NP: nitrophenol, DHCA: dihydrocaffeic acid, DOPA: dihydroxyphenylalanine, DHBA: dihydroxybenzoic acid, PHG: phloroglucinol, GA: gallic acid, 5-HODA: 5-hydroxydopamine, a.u.: arbitrary units.



**Supplementary Fig. 6.** Precursor-dependent behavior of surface-initiated pigment generation through the PAINT approach. The membrane without HRP was simply immersed in the precursor solution, showing the intrinsic color of unreacted precursors. The membrane with HRP contained four 1  $\mu$ L-sized spots of 0.001, 0.01, 0.1 and 1 U/mL HRP, which were visualized by in situ generated and attached pigments.



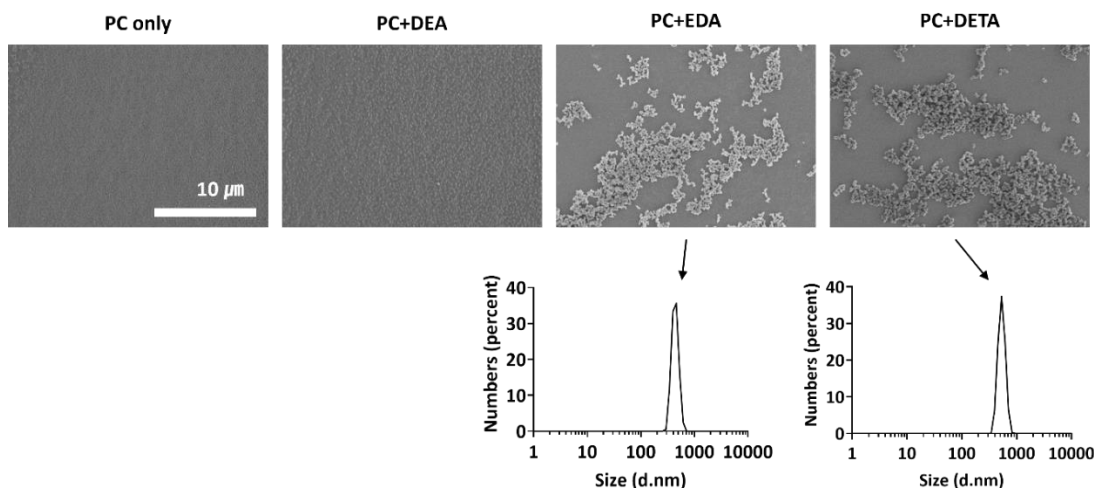
**Supplementary Fig. 7.** UV-visible spectra of diffused pigment in solution during surface-initiated pigmentation of various precursors (red: incubation w/o HRP-spotted membrane, green: incubation with HRP-spotted membrane). a.u.: arbitrary units.



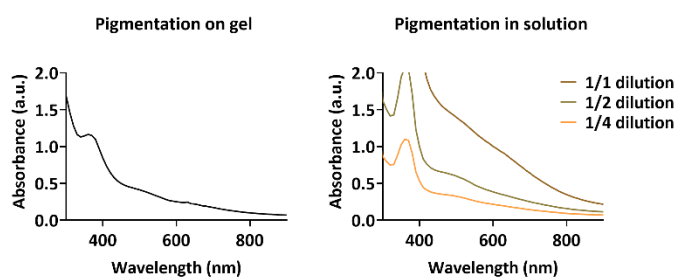
**Supplementary Fig. 8.** Plausible structures of water-soluble intermediates identified from the PC-based pigmentation with three different amine additives through HPLC-MS analysis.

R-NH <sub>2</sub>	Species	Elution time	m/z	Catechol	Primary amine	Schiff-base
DETA	1	1.5 min	366	2	4	3
	2	2.0 min	366	2	4	3
	3	2.7 min	566	4	4	4
EDA	4	3.6 min	480	4	4	4
DEA	5	1.7 min	358	2	2	-

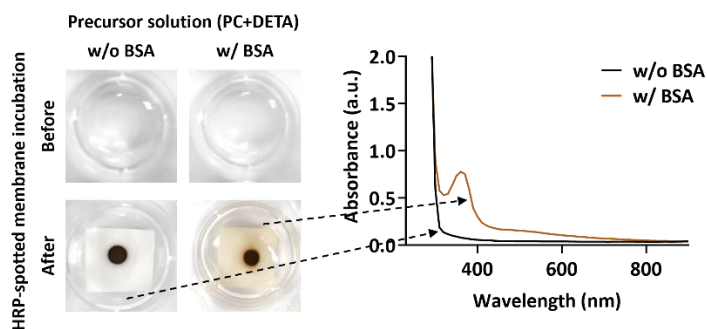
**Supplementary Table 1.** HPLC-MS analysis of water-soluble intermediates identified from the PC-based pigmentation with three different amine additives.



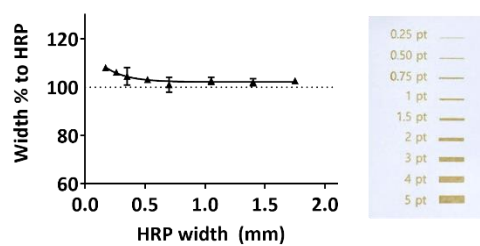
**Supplementary Fig. 9.** Aggregates formed during the solution-phase reaction of PC and amine additives with HRP. The aggregates were thoroughly filtered out before HPLC-MS analysis.



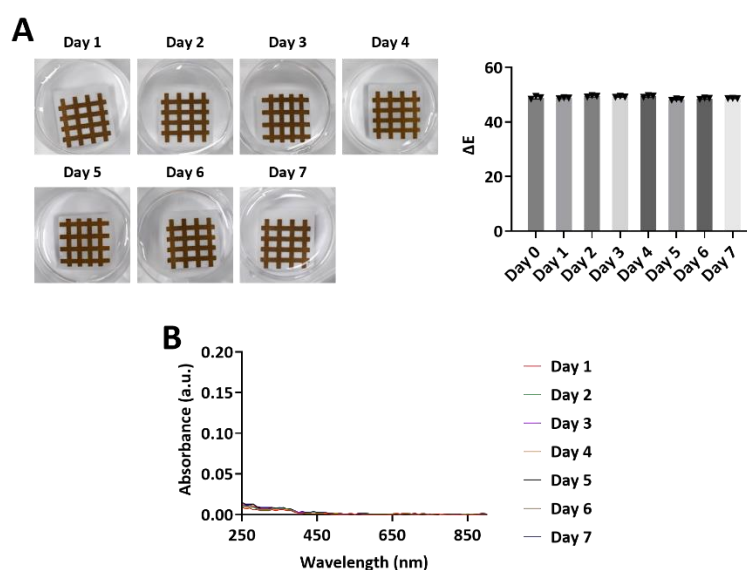
**Supplementary Fig. 10.** Comparison of solution-synthesized pigment and the pigment fabricated on a gel by PAINT approach. a.u.: arbitrary units.



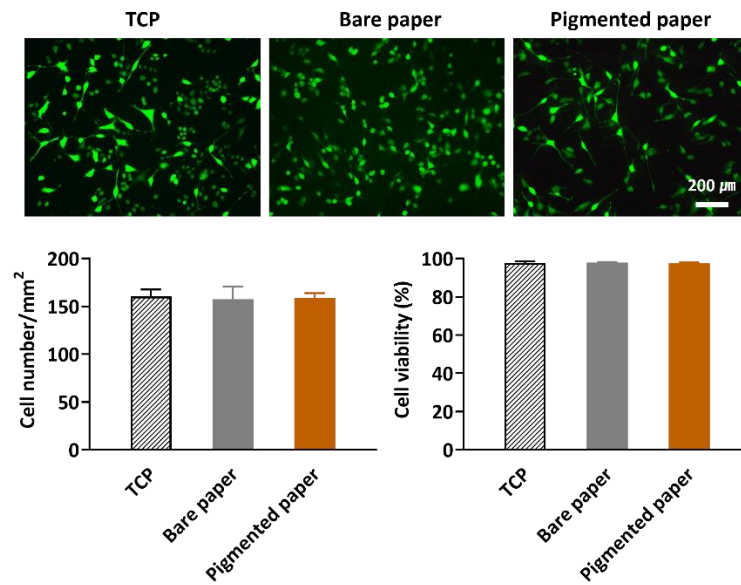
**Supplementary Fig. 11.** UV-visible spectrum of pigment that had diffused into the solution during surface-initiated pigment fabrication by the PAINT approach in the presence of BSA in the precursor solution. a.u.: arbitrary units.



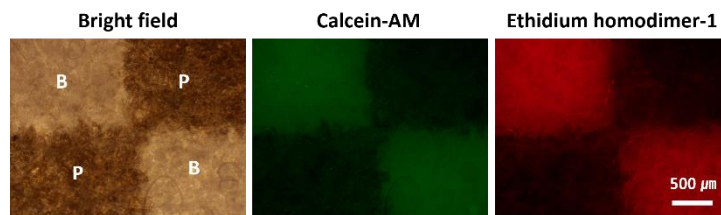
**Supplementary Fig. 12.** Width expansion% of PC-based pigment from each inkjet-printed HRP pattern with different widths ranging from 0.2 to 1.75 mm.



**Supplementary Fig. 13.** Intensity monitoring of PC/DETA pigment (A) and the detection of leachable products formed (B) during the incubation of printed PC/DETA pigment maintained in 1×PBS (pH 7.4) at 37 °C for 7 days. a.u.: arbitrary units.



**Supplementary Fig. 14.** Comparison of cell adhesion and viability of conventional tissue culture plate (TCP), bare A4 paper and pigmented A4 paper.



**Supplementary Fig. 15.** Attenuation of background fluorescence from the pigmented region of A4 paper.



	Chemical	Purity	Vendor
1	p-Phenylenediamine (p-PDAM)	98%	Sigma Aldrich
2	o-Phenylenediamine (o-PDAM)	98%	Sigma Aldrich
3	3-Nitrophenol (3-NP)	99.50%	Sigma Aldrich
4	2-Aminophenol (2-AP)	99.90%	Sigma Aldrich
5	4-Aminophenol (4-AP)	100%	Sigma Aldrich
6	4-Ethylcatechol (4-EC)	95%	Sigma Aldrich
7	4-Nitrocatechol (4-NC)	99.10%	Sigma Aldrich
8	3,4-Dihydroxy-L-phenylalanine (DOPA)	100%	Sigma Aldrich
9	2,4-Dihydroxybenzoic acid (2,4-DHBA)	99.90%	Sigma Aldrich
10	Pyrocatechol (PC)	99%	Sigma Aldrich
11	Dopamine hydrochloride (DA)	98%	Sigma Aldrich
12	3,4-Dihydroxyhydrocinnamic acid (3,4-DHCA)	98%	Sigma Aldrich
13	3,5-Dihydroxybenzoic acid (3,5-DHBA)	99.90%	Sigma Aldrich
14	Chlorogenic acid	98%	TCI
15	2,5-Dihydroxybenzoic acid (2,5-DHBA)	98%	Sigma Aldrich
16	Resorcinol	99%	Sigma Aldrich
17	Phloroglucinol (PHG)	99.70%	Sigma Aldrich
18	Gallic acid (GA)	99.20%	Sigma Aldrich
19	5-Hydroxydopamine hydrochloride (5-HDA)	98%	Sigma Aldrich
20	6-Hydroxydopamine hydrochloride (6-HDA)	100%	Sigma Aldrich
21	Catechin hydrate	99%	Sigma Aldrich
22	Pyrogallol (PG)	100%	Sigma Aldrich
23	3,3',5,5'-Tetramethylbenzidine (TMB)	100%	Sigma Aldrich
24	2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS)	100%	Sigma Aldrich
25	3, 3-Diaminobenzidine (DAB)	97.50%	Sigma Aldrich
26	Hydrogen peroxide (H <sub>2</sub> O <sub>2</sub> )	30wt. % in H <sub>2</sub> O	Sigma Aldrich
27	Diethylenetriamine (DETA)	99.90%	Sigma Aldrich
28	Diethylamine (DEA)	99.90%	Sigma Aldrich
29	Ethylenediamine (EDA)	99.92%	Sigma Aldrich
30	Trifluoroacetic acid (TFA)	99.00%	Sigma Aldrich
31	Acetonitrile (ACN)	100%	Sigma Aldrich

**Supplementary Table 2.** Purities of purchased materials.