Supplementary Materials: Using Laccases in the Nanoflower to Synthesize Viniferin

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1. pH stability

The pH stability of the free laccase and the immobilized laccase was determined by incubating the enzyme in different buffers (pH 3.0-8.0) for 6 h at 35 °C. The buffers used were Na_2HPO_4–sodium citrate buffer (pH 3.0-8.0). The immobilized laccase was collected by centrifugation (3500 g for 3 min) and air-dried. The residual specific activities of free or immobilized laccase were then determined at its optimum pH according to the assay in Section 3.4 described in the text.

As can be seen from Figure S1, there is no significant difference in the specific activities of immobilized laccase incubated by different pH buffers ranging from 3 to 7 ($p > 0.05$). In contrast, the specific activity of free laccase treated by buffer (pH 4.0) is significantly higher than that of the cases incubated by other buffers (pH 3–8) ($p < 0.05$). After incubation by buffers (pH 3.0 and pH 5.0), free laccase has significantly higher specific activity compared with the cases treated by different buffers in the pH range 6–8 ($p < 0.05$). The specific activity of free laccase incubated by buffer (pH 8.0) is significantly lower than that of the cases treated by the buffers (pH 6.0 and pH 7.0) ($p < 0.05$). The above-mentioned results demonstrate that laccase in the nanoflower is less sensitive to pH change compared with the free laccase.

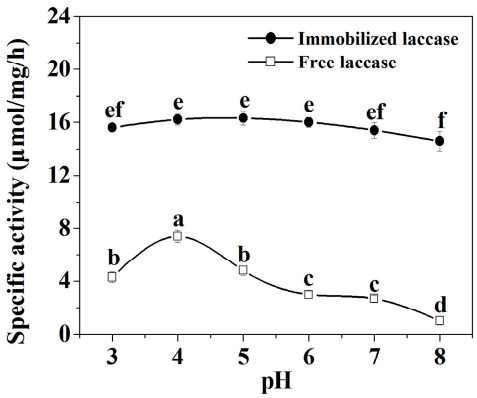


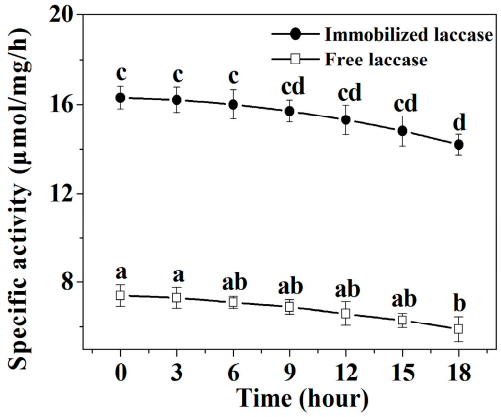
Figure S1. pH stability of free or immobilized laccase tested at different pH values using resveratrol as substrate. Post hoc all pairwise comparisons for the specific activities of free laccase or immobilized laccase at different pH values were performed using Tukey's test ($p < 0.05$). Means with the same letter are not significantly different. Letters (a, b, c, d, e, f) identify different statistically significant groups (Tukey's test, $p < 0.05$).

2. Thermal stability

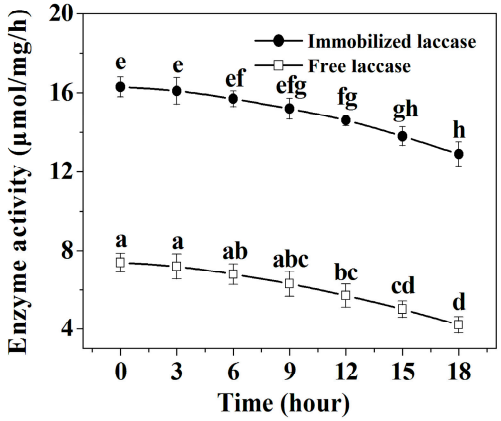
The time courses of the irreversible thermoinactivation were measured by incubating each enzyme (free or immobilized) in its optimal pH at 45 °C, 55 °C, or 65 °C, respectively. At the regular intervals, samples were removed and cooled on ice and subsequently, the remaining specific activity was determined according to the assay in Section 3.4 described in the text.

The results show that the specific activities of free laccase and immobilized laccase decrease with the increasing incubation time at 45 °C, 55 °C, or 65 °C, respectively (Figure 2). In Figure S2a, there is a significant difference in the specific activities of free laccases between the samples incubated by the buffer (pH 4.0) for 6 h and 18 h at 45 °C ($p < 0.05$). On the contrary, the specific activities of immobilized laccase incubated by the buffer (pH 5.0) for 6 h and 18 h are not

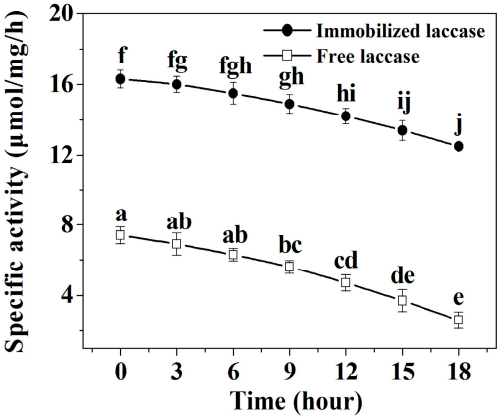
distinguishable from one anther at 45 °C ($p > 0.05$). Furthermore, at 65 °C, the specific activity of free laccase incubated by the buffer (pH 4.0) for 6 h is significantly higher than that of the case incubated for 12 h ($p < 0.05$), while immobilized laccase incubated by the buffer (pH 5.0) for 6 h does not have significant higher specific activity compared with the case incubated for 12 h ($p > 0.05$) (Figure S2c). These results suggest that the specific activity of laccase in the nanoflower decreases at a slower rate than that of the free one with the increase of the incubation time, which implies that the immobilization of laccase in the nanoflower might preserve the tertiary structure of the protein and protect laccase from disassembling of the active center caused by the diminution of non-covalent forces at higher temperatures [1].



(a)



(b)



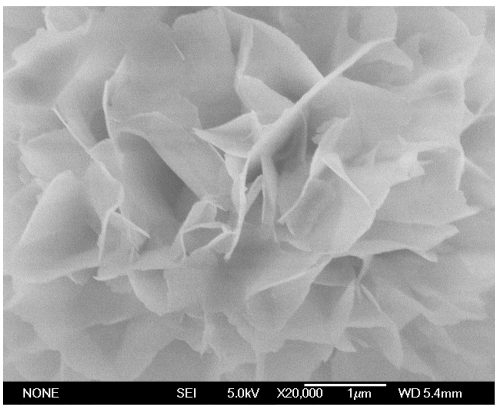
(c)

Figure S2: Thermal stability at 45 °C (a), 55 °C (b), and 65 °C (c) of free laccase and immobilized laccase tested in different time intervals using resveratrol as substrate. Post hoc all pairwise comparisons for the specific activities of free laccase or immobilized laccase at different time intervals were performed using Tukey's test ($p < 0.05$). Means with the same letter are not significantly different. Letters (a, b, c, d, e, f, g, h, i, j) identify different statistically significant groups (Tukey's test, $p < 0.05$).

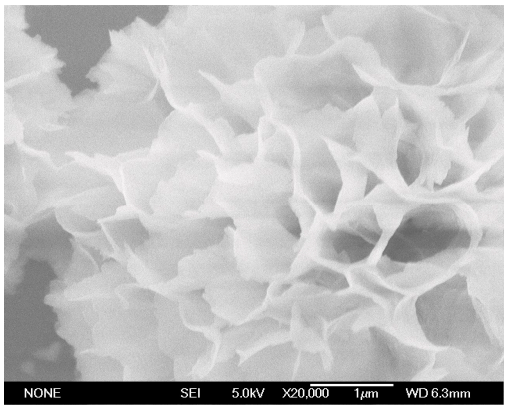
3. NMR analysis

The spectra are listed as follows. 1H-NMR (500 MHz, CD_3COCD_3) δ: 8.43 (5H, brs), 7.22 (2H, d, J = 8.5 Hz), 7.19 (2H, d, J = 8.5 Hz), 6.94 (1H, d, J = 16 Hz), 6.85 (2H, d, J = 8.5 Hz), 6.77 (2H, d, J = 8.5Hz), 6.76 (1H, d, J = 2.0 Hz), 6.73 (1H, d, J = 16.0 Hz), 6.36 (1H, d, J = 2.0 Hz), 6.29 (brs, 3H), 5.43 (1H, d, J = 5.5 Hz), 4.51 (1H, d, J = 5.5 Hz).

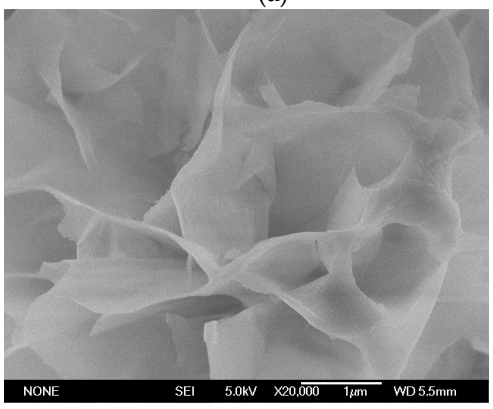
4. The SEM Images of Laccase Incorporated Nanoflowers at Different Reaction Conditions



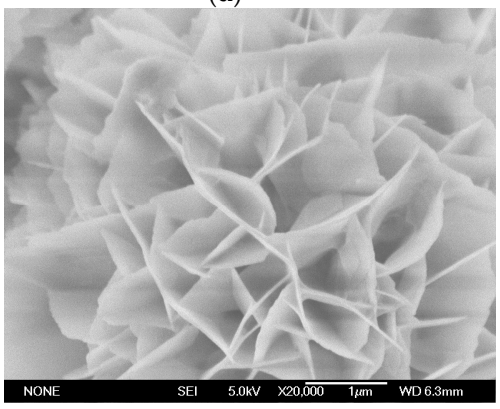
(a)



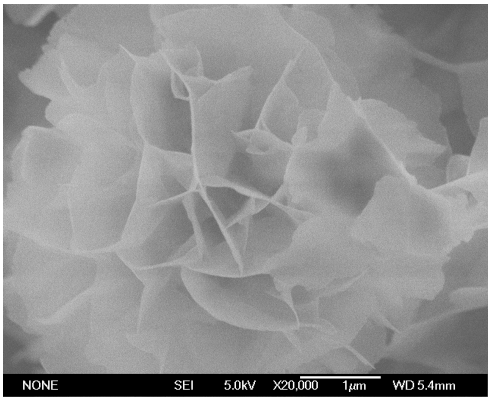
(d)



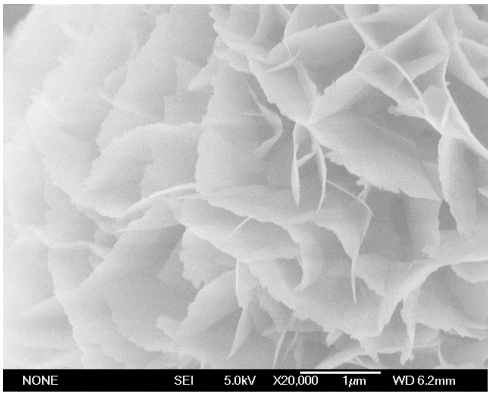
(b)



(e)



(c)



(f)

Figure S3. SEM images of laccase-incorporated nanoflower at 35 °C: (**a**) pH = 3, (**b**) pH = 5, and (**c**) pH = 8 or different temperatures at pH 5.0: (**d**) 5 °C, (**e**) 35 °C, and (**f**) 65 °C.

Table S1. The specific activity of free and immobilized laccase at 4 °C and 25 °C.

Temperature (°C)	Specific activity (μmol/mg/h)	
4 °C	2.1±0.56 [a]	12.0±0.63 [b]
25 °C	5.0±0.7 [a]	13.7±0.38 [b]

[a] free laccase; [b] laccase in the nanoflower

References

1. Murphy, K.P. *Protein Structure, Stability, and Folding*; Springer: Berlin/Heidelberg, Germany, 2001.