catalysts

Supplementary information:

Enzyme Immobilization on Stainless Steel Fleece and Its Mass Transfer Enhancement of Enzymatic Catalysis in a Rotating Packed Bed Reactor

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1. Calculations of the loading absorption capacity

In the test of enzyme loading on metal carriers, the BSA method was chosen. A standard curve was obtained, which could be represented by y = 0.0009x and R^2 = 0.9925. The linear dependence was reliable and can be used for testing the loading amount of protein.

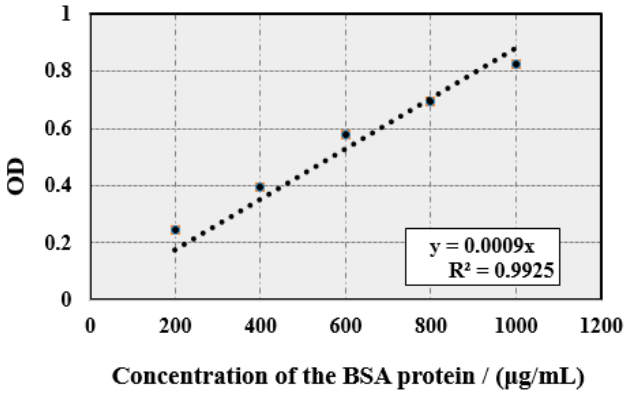

Figure S1. Standard curve of the BSA protein.

In the experiment of determining the protein loading absorption at the metal carrier, oleic acid was chosen to graft and epoxidize wire mesh, and it was catalyzed by CALB. According to the BSA standard curve, the protein concentration of CALB raw material liquid can be determined to be 13.175 mg/mL.

$$\text{Enzyme capacity } (\mu g/cm^2) = Ci \times \text{Immobilized recovery } (\%) \quad \text{(Equation 1)} \qquad (1)$$

The immobilized recovery rate follows Equation1. The area protein load of the mesh metal carrier (including the mesh aperture area) was 6.59 μg/cm².

2. Calculation of V_{max} and K_m

In the synthesis system of n-octyl laurate, the electrification of the substrate is ignored, so the catalytic reaction rate of the free enzyme conforms to the Michaelis-Menten equation, and the reaction rate formula is:

$$\frac{1}{V} = \frac{K_m}{V_{\max}} \frac{1}{[C_0]} + \frac{1}{V_{\max}} \quad \text{(Equation 2)} \tag{2}$$

In the reaction of producing an *n*-octyl laurate, keeping the metal carrier and resin consisted of the same enzyme loading amount. During the experiment, a 13.18 μg CALB protease weight immobilized enzyme (2 cm^2 immobilized enzyme on metal and 5 mg resin-immobilized enzyme) was added to 3.5 mL subtracts liquid at different concentrations, respectively. The reaction was carried out in a stirred reactor at 40 °C and 800 rpm. The double-reciprocal fitting formula for the data of concentration and reaction rate of the mesh immobilized CALB was Y = 0.0048x + 0.09834. The linear correlation was R^2 = 0.9995 which has high confidence. Under the same conditions, the double-reciprocal fitting formula for the concentration and reaction rate of the resin immobilized with enzyme working as the control group was Y = 0.0031x + 0.1921. (Shown in figure S2). The linear correlation of the curve was R^2 = 0.9895, which also provides high confidence. The two immobilized enzyme fitting formulas have a high reliability. The intrinsic dynamic parameters are shown in Table 2. The double-reciprocal fitting formula for the data of concentration and reaction rate of the mesh immobilized FAH was Y = 0.00266x + 1.053. The linear correlation was R^2 = 0.9972 which has high confidence. The double-reciprocal fitting formula for the concentration and reaction rate of the resin immobilized with the enzyme working as the control group was Y = 0.0176x + 1.5913. The linear correlation of the curve was R^2 = 0.9907, which also provides high confidence.

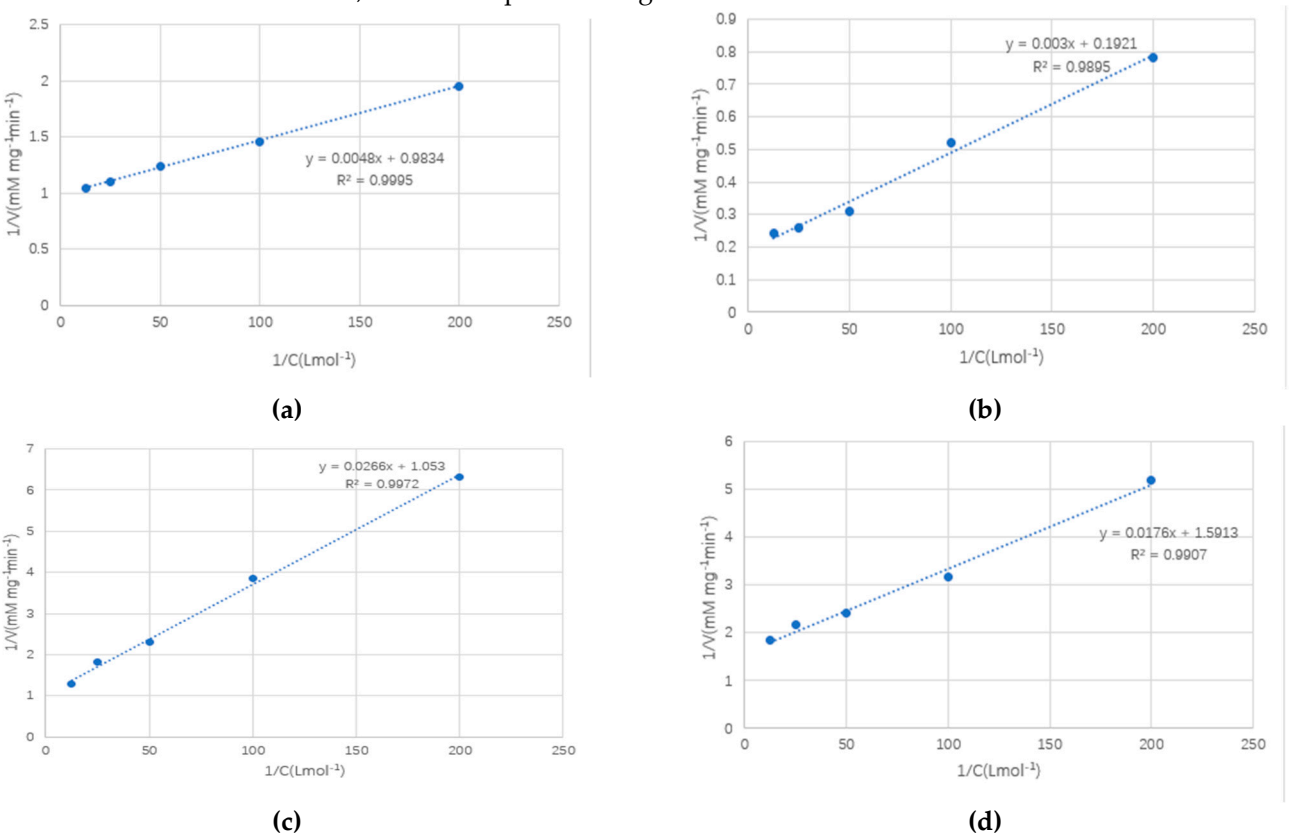

Figure S2. Fitting curve of double reciprocal of concentration and rate of enzyme（a) CALB lipase on stainless-steel net (b) CALB lipase on D3520 Resin（c) FAH on Stainless-steel net (d) FAH on D3520 Resin.

3. Gas Chromatography

The reaction products were analyzed by GC (GC-2010 plus, Shimadzu, Tokyo, Japan) equipped with DB-1 ht (30 m × 0.25 mm × 0.10 μm). The contents of products were calculated with the method of area normalization. The concentration of products were determined using the external standard method. All measurements were conducted in triplicate. Compared with the standard linoleic acid, the remain time of linoleic acid was validated at 13.74 min. Along with the reaction time elongating, the peak areas of substrates decreased while the products' peak areas increased. The product's structure was determined by ^{1}H-NMR in the next step.

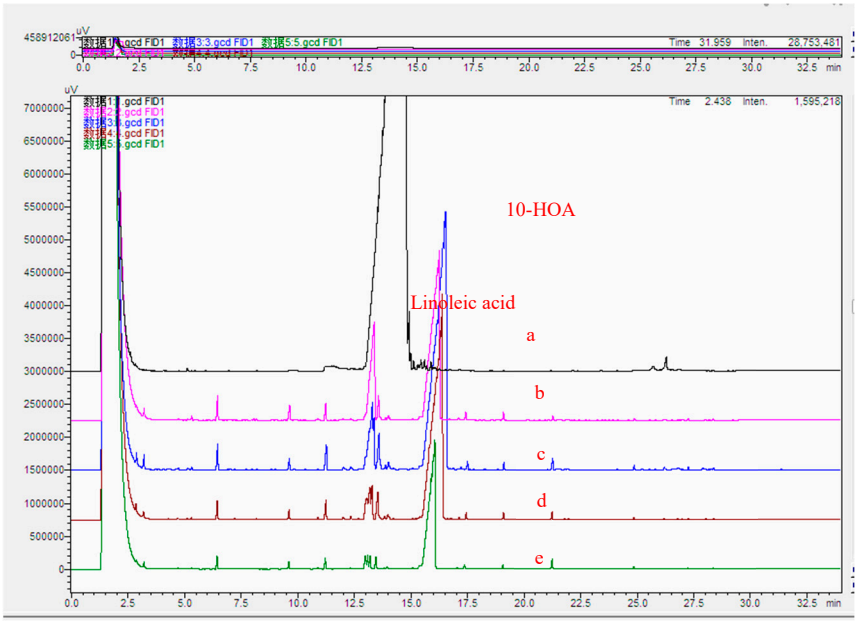

Figure S3. Gas Chromatography of 10-HOE (**a**) Standard Linoleic acid (**b**) reaction for 1 h (**c**) reaction for 2 h (**d**) reaction for 4 h (**e**) reaction for 10 h.

4. SDS-PAGE for FAH

SDS results shown that the FAH is correctly expressed with a molecular weight of 67.6 kDa. The first brand was FAH. As it shown in the second, third and fourth brand, the target protein remained after the purification and other protein disappeared.

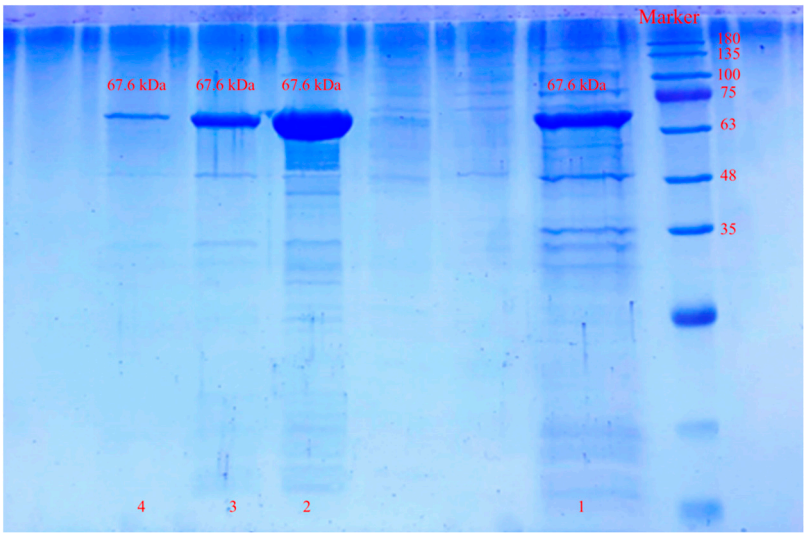

Figure S4. SDS-PAGE result of FAH 1. FAH before His-tag purification; 2. FAH after first time of purification; 3. FAH after second times of purification; 4. FAH after third times of purification;.

5. ^{1}H-NMR characters for 10-hydroxy-*cis*-12-octadecenoicacid

According to the chemical shifts of each proton and the intensity of peaks, ^{1}H-NMR was validated, and the product is 10-hydroxy-*cis*-12-octadecenoicacid.

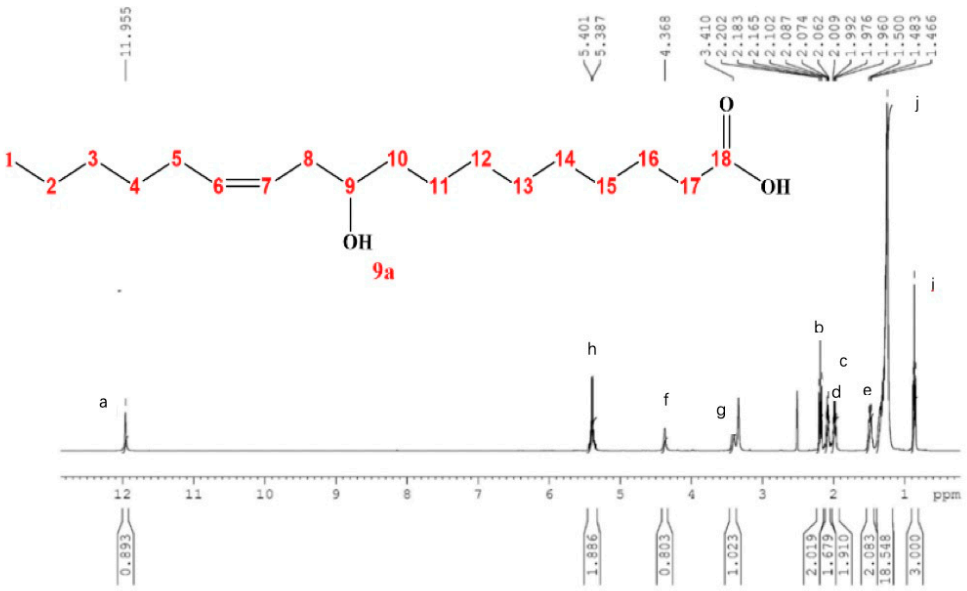

Figure S5. Identification of the hydration product by ^{1}H-NMR.

Table S1. Proton NMR Signals and molecular Assignments for 10-hydroxy-*cis*-12-octadecenoicacid.

Carbon number	Proron type	Chemical Shift (ppm)
1	i	0.88
2~4,10~15	j	1.46~1.50
5	d	2.16
6~7	h	5.38~5.40
8	c	2.18
9a	f	4.36
9	g	3.41
16	e	1.50
17	b	2.20
18	a	11.95