Lipase/tannic acid magnetic hydrogel microspheres and its continuous catalytic application

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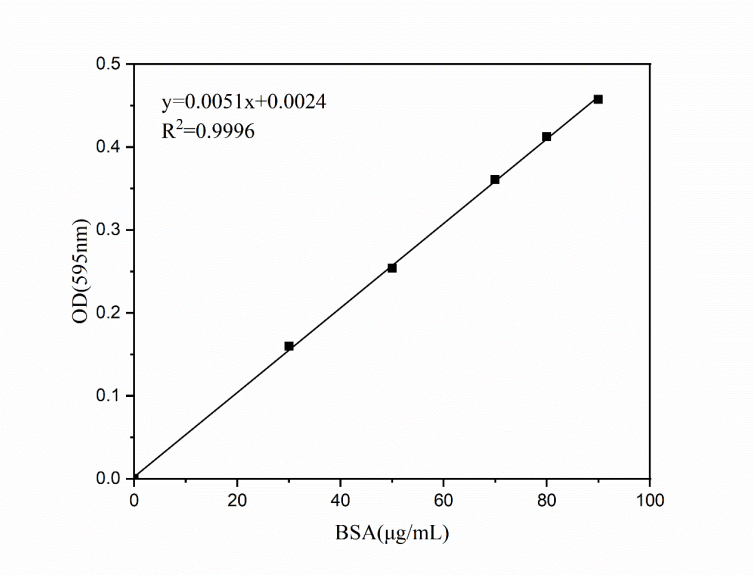

Fig. S1 The protein standard curve of BSA

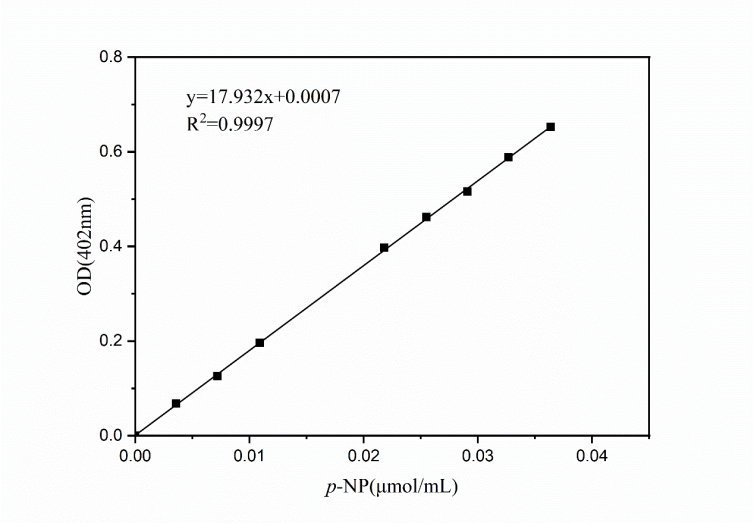

Fig. S2 The standard curve of *p*-NP

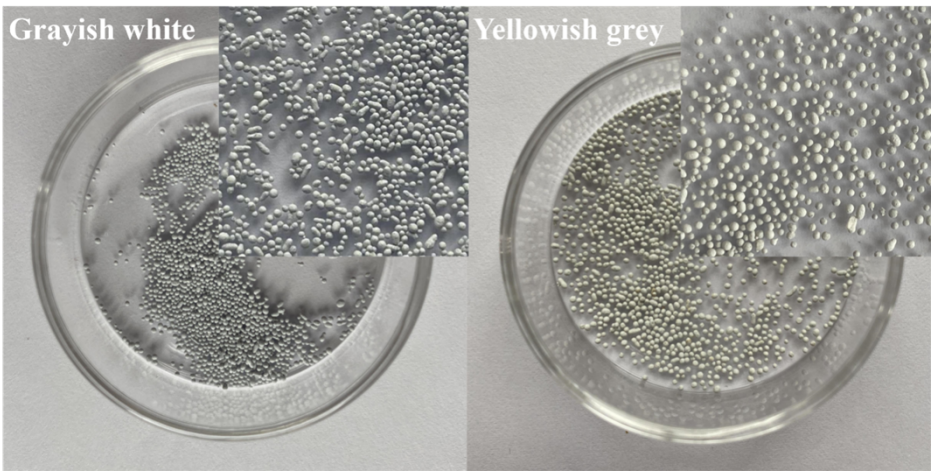

Fig.S3 Photographs of PM and PTAM

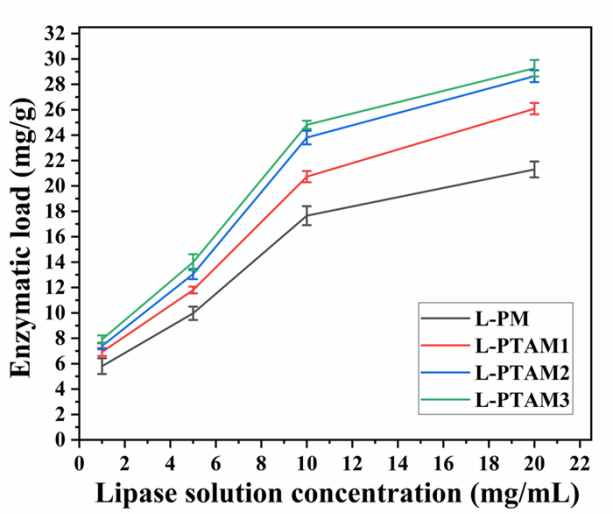

Fig.S4 The effect of PM, PTAM1, PTAM2 and PTAM3 on lipase loaded in lipase solutions with different concentrations (n=3)

Table S1. The EDS data of elements C, N, O, S and Fe content in the L-PM and L-PTAMs

Sample/Element	L-PM	L-PTAM1	L-PTAM2	L-PTAM3
	Wt%			
C	54.28	51.57	55.95	53.08
N	14.47	15.89	10.86	12.22
O	30.49	31.68	32.00	33.37

S	0.17	0.32	0.44	0.49
Fe	0.89	0.63	0.76	0.84
Total	100.00	100.00	100.00	100.00

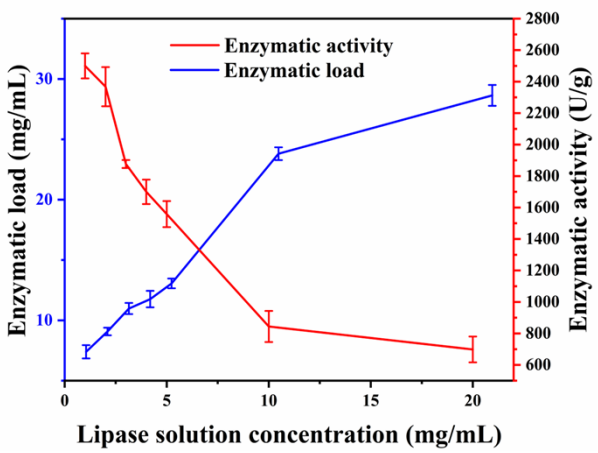

Fig. S5. The enzymatic load and enzymatic activity of L-PTAM2 at different concentrations of lipase solution on (n = 3)

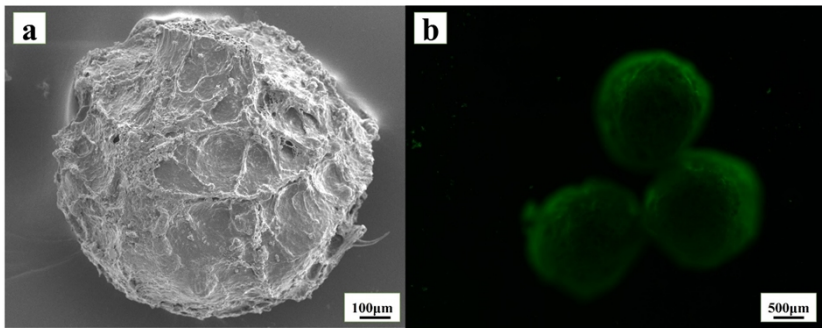

Fig.S6. (a) The SEM image of L-PTAM2-20 after 20 cycles; (b) The fluorescence image of L-PTAM2-20 after 20 cycles

Detailed calculation of kinetic parameters:

The Lineweaver-Burk double-reciprocal plot method was used to determine the free lipase and L-PTAM2 Michaelis-Menten constants (K_m) and maximum reaction velocities (V_{max}) values. The *p*-NPP substrate was used at various concentration from 0.165 mM to

1.815 mM, and the amount of free lipase used was identical to that on the L-PTAM2.

Table S2 Reaction rates (V) in different substrate concentrations (S)

p-NPP (μmol/mL)		0.165	0.495	0.825	1.155	1.485	1.815
V	Free lipase	0.2526	0.7672	1.1790	1.4571	1.6889	1.8096
(U/mg)	L-PTAM2	0.1937	0.3621	0.7401	1.5347	1.7325	2.1017

Table S3 The 1/S and 1/V

1/S		0.5510	0.6734	0.8658	1.2121	2.0202	6.0606
1/V	Free lipase	0.5526	0.5921	0.6863	0.8482	1.3034	3.9582
	L-PTAM2	0.4758	0.5772	0.6516	1.3511	2.7618	5.1625

Use 1/ S and 1/ V to plot:

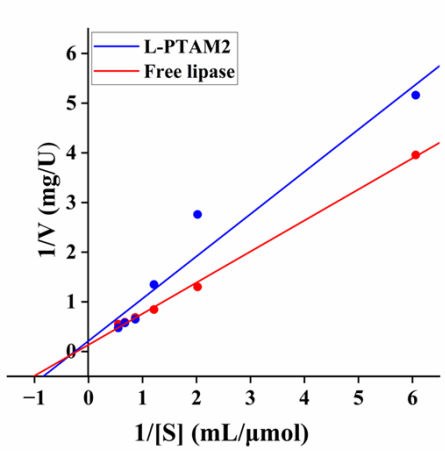

Fig. S7 The Lineweaver-Burk plot of free lipase and L-PTAM2

Calculated as follows：

$$\frac{1}{V} = \frac{Km}{Vmax} \times \frac{1}{[S]} + \frac{1}{Vmax}$$

The *Km* and *Vmax* values of free lipase and L-PTAM2 were btained by calculating the curve intercepts on X-axis and Y-axis, and shown as follow.

Table S4 The kinetic parameters of free lipase and L-PTAM2

Sample	V_{max}(U/mg)	K_m (μmol/mL)
Free lipase	4.6466	3.9550
L-PTAM2	7.3174	4.5775

Thin layer chromatography purification analysis

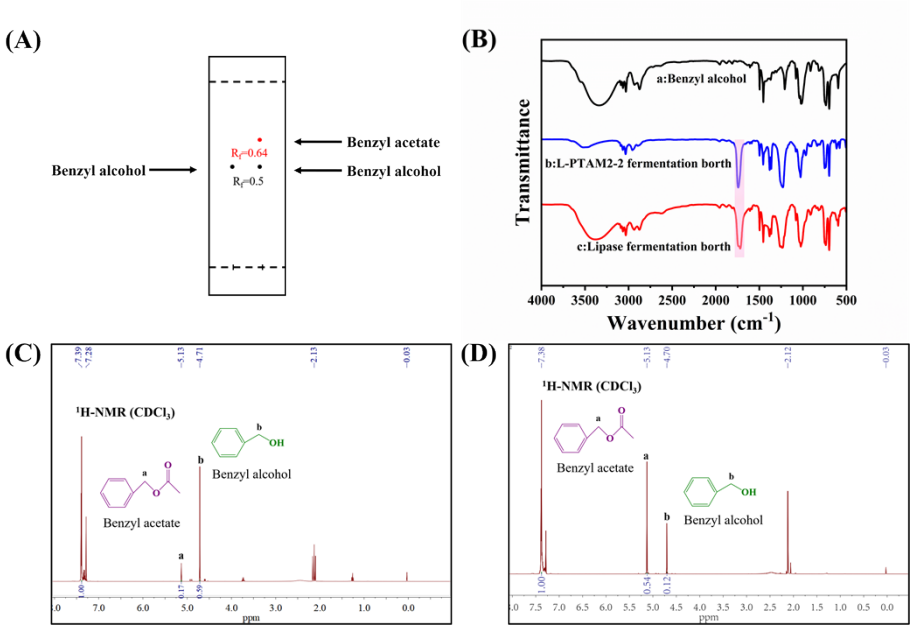

Fig. S8. (A) TLC schematic diagram of benzyl alcohol and benzyl acetate; (B) The FT-IR spectra of (a) benzyl alcohol, (b) benzyl acetate product catalyzed by L-PTAM2-2, and (c) lipase fermentation borth (C) ^{1}H NMR (400 MHz) specturm of the benzyl acetate product catalyzed by lipase fermentation broth; (D) ^{1}H NMR (400 MHz) specturm of the benzyl acetate product catalyzed by L-PTAM2 fermentation broth.

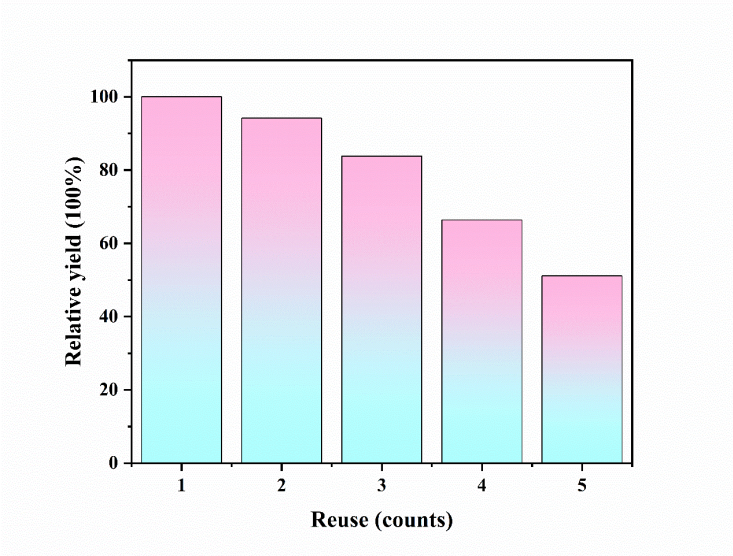

Fig. S9. The relative yield of benzyl acetate in the continuous reaction

The uniform mixed solution of 2 g of benzyl alcohol and 20 g of vinyl acetate was dropped into the reactor at a flow rate of 3ml/min, and the mixed solution was kept in contact with L-PTAM2. After the reaction, the valve was opened to collect the product to test the reusability of L-PTAM2.

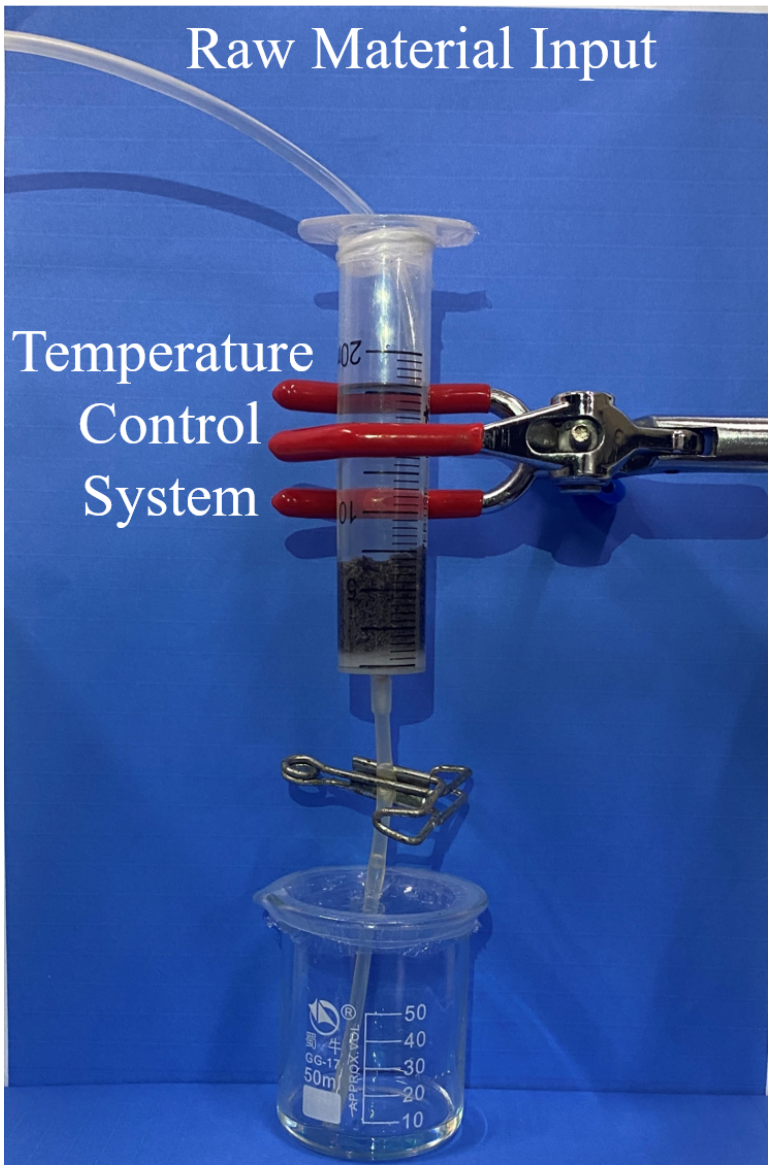

Fig.S10. The photo of the experimental device for continuously catalyzing the synthesis of benzyl acetate with L-PTAM2 as catalyst.