Electronic Supplementary Material (ESI) for New Journal of Chemistry.

Supporting Information

Feed ratios of the NPC

NPC2: lipase-$Cu_3(PO_4)_2$•$3H_2O$nanoflowers (10 mg), 10 wt% PVA aqueous solution (0.400 g), and 5 wt% chitosan aqueous solution (0.100g) ;

NPC3: lipase-$Cu_3(PO_4)_2$•$3H_2O$nanoflowers (10 mg), 10 wt% PVA aqueous solution (0.266 g), and 5 wt% chitosan aqueous solution (0.066 g) ;

NPC4: lipase-$Cu_3(PO_4)_2$•$3H_2O$nanoflowers (10 mg), 10 wt% PVA aqueous solution (0.200 g), and 5 wt% chitosan aqueous solution (0.050 g) ;

NPC5: lipase-$Cu_3(PO_4)_2$•$3H_2O$nanoflowers (10 mg), 10 wt% PVA aqueous solution (0.160g), and 5 wt% chitosan aqueous solution (0.040 g) .

Thecalculationof activity recovery:

$$Activity\ recovery\ \% = \frac{Immobilized\ enzyme\ activity + Unimmobilized\ enzyme\ activity}{the\ total\ activity\ of\ \ free\ enzyme}$$

(2) Immobilized enzyme activity = the weight of the obtained nanoflowers (0.1059 g) × the ratio of enzymes to weight in nanoflowers (9.88%)×specific activity of enzymatic protein in nanoflowers (245.46U/g)=2.57U

Unimmobilized enzyme activity=the weight ofunimmobilizedfree enzyme content (0.225g-0.1059g×9.88%)×specific activity of free enzyme (97.28U/g)=20.87U

The total activity of free enzyme= the total weight of immobilized free enzyme (0.225g) ×specific activity of free enzyme (97.28U/g)=21.88U

Activity recovery can be amended to 107.12%.

Table S1.The weight percent of lipase inlipase-$Cu_3(PO_4)_2$•$3H_2O$ nanoflowers.

Enzyme concentration (mg/mL)	Value of pH	Nanoflower (g)	$Cu_3(PO_4)_2$ (g)	$Cu_3(PO_4)_2$•$3H_2O$ (g)	Weight percentage of enzyme (%)	The average value of weight percentage (%)
0.25	7.4	0.2004	0.1579	0.1803	10.05	
0.25	7.4	0.2006	0.1582	0.1806	9.97	9.98±0.06
0.25	7.4	0.1998	0.1576	0.18	9.92	

Table S2. Immobilization yield and activity recovery of nanoflowers.

Times of immobilization	The weight of the obtained nanoflowers（g）	The ratio of enzymes to weight in nanoflowers(%)	Immobilization yield(%)	Activity recovery after six times immobilization(%)
1	0.1059	0.0988	4.65	107.12
2	0.0987	0.0937	8.76	113.39
3	0.0954	0.0892	12.54	119.15
4	0.0916	0.0847	15.99	124.40
5	0.0872	0.0804	19.11	129.15
6	0.0855	0.0765	22.01	133.58

Table S3. The enzymatic activities of free lipase, nanoflowers and NPC1-NPC5.

Enzyme	Enzyme activity (U/mg)	Relatively activity (%)
Free lipase	97.28±6.57	100±6.75
Nanoflowers	246.76±8.00	253.66±8.22
NPC1	130.77±7.98	134.43±8.20
NPC2	129.37±9.96	132.99±10.23
NPC3	146.12±6.54	150.21±6.72
NPC4	120.34±2.17	123.70±2.23
NPC5	82.42±2.61	84.72±2.68

Table S4. The enzymatic activities of nanoflowers andNPC3 in recycle use

Run Recycles	Enzyme activity(U/g)	
	Nanoflowers	NPC3
1	234.47±2.64	133.30±10.61
2	124.90±3.23	93.62±9.11
3	90.28±4.77	72.56±6.63
4	31.70±2.24	78.12±4.49
5		46.64±4.17
6		51.42±4.59
7		40.46±0.12

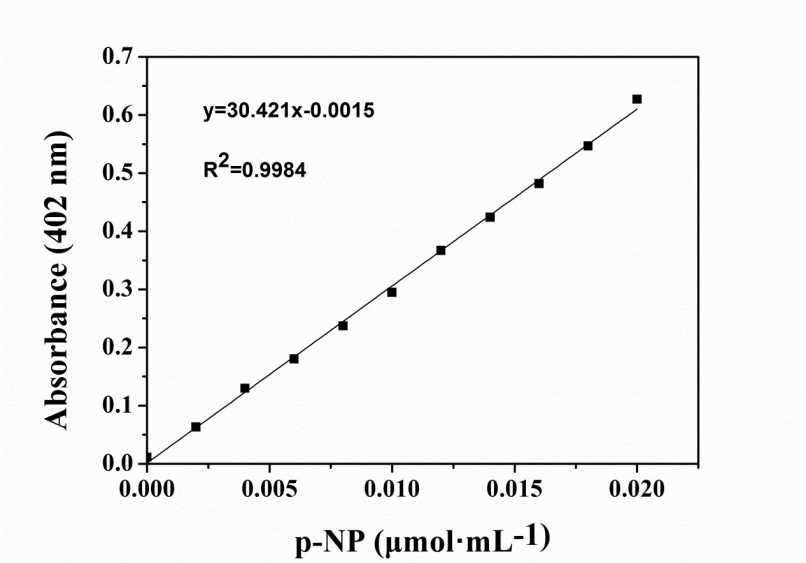


Figure S1. Standard curve of p-NP

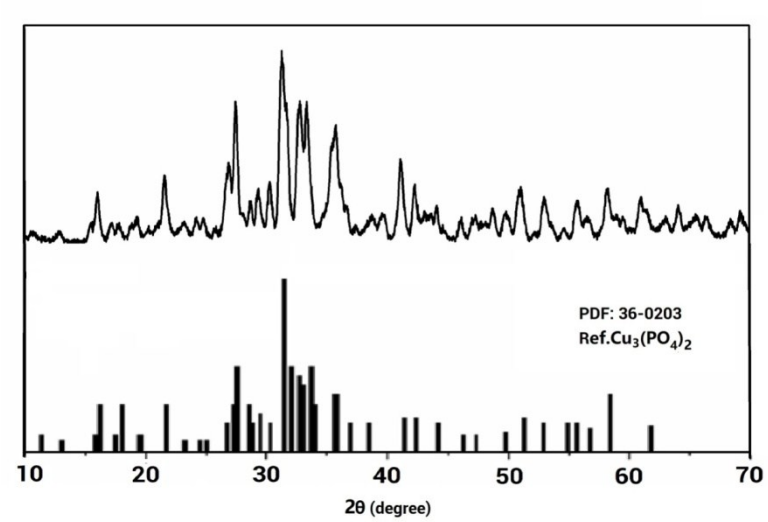
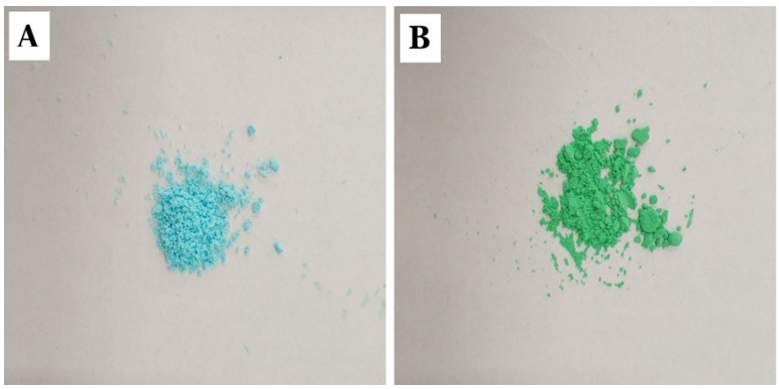


Figure S2. XRD patterns of of hybrid nanoflowers after 700°C calcination and JCPD card No. 36-0203.



FigureS3. (A) Photograph of lipase-$Cu_3(PO_4)_2$ hybrid nanoflowers, (B) Photograph of

hybrid nanoflowers after 700°C calcination.

Detailed calculation of kinetic parameters：

The kinetic parameters (K_m and V_{max}) of free lipase, lipase-$Cu_3(PO4)_2$•$3H_2O$ nanoflowers and NPC3 were calculated by the Lineweaver-Burk double-reciprocal plot method. The concentration of p-NPP (as substrate) was set from 0.1 mM to 1.0 mM and the enzyme concentration was 1 mg/mL. The enzymatic reaction was carried at 37 ^{O}C and pH 7.4 in PBS.

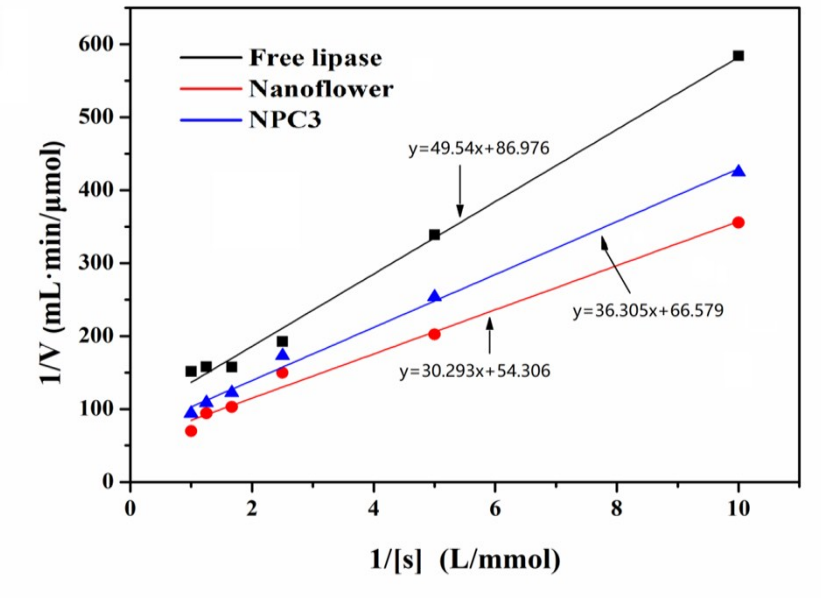
Reaction rates (V)in different substrate concentrations (S)：

p-NPP(mM)		1.0000	0.8000	0.6000	0.4000	0.2000	0.1000
Initial rate of reaction (μmol/mL·min)	free-lipase	0.0066	0.0063	0.0063	0.0052	0.0030	0.0017
	nanoflower	0.0143	0.0106	0.0097	0.0067	0.0049	0.0028
	NPC3	0.0106	0.0092	0.0082	0.0058	0.0039	0.0024

The reciprocal of S and V.

1/S		1.0000	1.2500	1.6667	2.5000	5.0000	10.0000
1/V	free-lipase	151.5954	157.8534	157.5045	192.7939	338.9105	584.1282
	nanoflower	69.7855	94.3435	102.7661	149.7421	202.4535	355.5102
	NPC3	93.9590	108.8145	122.4079	173.0846	253.8536	424.8780

Use the reciprocal of S and V to plot：



Calculated as follows：

$$\frac{1}{V} = \frac{K_m}{V_{max}} \times \frac{1}{[S]} + \frac{1}{V_{max}}$$

V is the initial reactive rate (mmol/L min); V_{max} is the maximal velocity of the reaction (mmol/L min); [S] is the initial substrate concentration(mmol/·L); and K_m is the Michaelis–Menten constant(mmol/L).

The K_m and V_{max} values for the three forms of lipase were obtained by calculating the intercept of the curves on the X-axis and Y-axis, and shown as follow.

Types of enzymes	Km(mmol/L)	Vmax(mmol/L·min)	Vmax/km
free-lipase	0.5696	0.0115	0.0202
lipase-$Cu_3(PO_4)_2$•$3H_2O$ nanoflowers	0.5429	0.0184	0.0339
NPC3	0.5576	0.0150	0.0269

The enzymatic catalysisreaction: