Electronic Supplementary Material (ESI) for RSC Advances.

Superparamagnetic Fe_3O_4 Nanoparticles Modified by Water-soluble and Biocompatible Polyethylenimine for Lipase Immobilization with Physical and Chemical Mechanisms

1. Effect of lipase amount added on the activity of ICRL

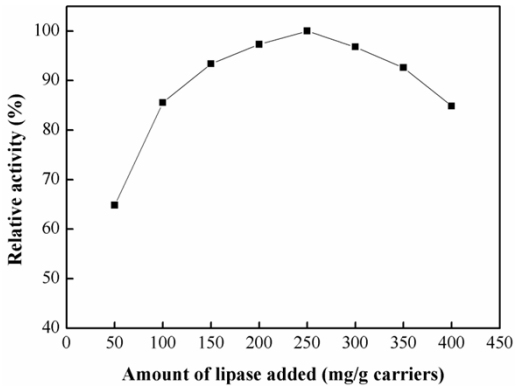

Figure s-1 (a) Effect of lipase amount added on the activity of C-ICRL

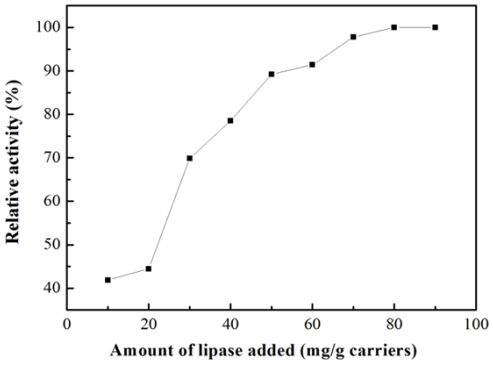

Figure s-1 (b) Effect of lipase amount added on the activity of I-ICRL

The influence of lipase amounts added on the activities of ICRL was studied, and the result is shown in figure s-1 (covalently immobilized CRL was marked as C-ICRL, while ICR immobilized with only ionic exchange was marked as I-ICRL). Immobilization reaction was tested in phosphate solution (0.1 M, pH = 7.0) at 30 °C for 5 h. The relative activities of C-ICRL increased obviously with the increasing amount of lipase until the relative activity of C-ICRL reached a maximum value, at which the amount of protein given was 250 mg/g support. After this, the relative activities of C-ICRL gradually decreased. The same phenomenon has been reported in our previous work. The reasons for this phenomenon might be considered as (1) the competition of reaction with aldehyde groups between CRL and other protein. For the lipase solution contained not only CRL but also other protein, when CRL immobilized on supports, other protein connected to the supports as well. Hence, the amounts of immobilized CRL were restricted; (2) an intermolecular steric hindrance was formed with higher enzyme loading, which caused decrease of enzymatic activity.

Although the relative activities of I-ICRL increased obviously with the increasing amount of lipase until the relative activity of I-ICRL reached a maximum value as well, at which the amount of protein given was 80 mg/g. After this, the relative activities of I-ICRL was invariable. This might because the physical adsorption is not strong enough to carry more lipase, or some lipases ran off when the supports were washed by phosphate solution after

immobilization. When the amount of lipase added was 50 mg/g, the loading amount of C-ICRL was 37.76 mg/g, while I-ICRL was 22.1 mg/g.

All the activities of C-ICRL and I-ICRL were lower than free CRL in the same conditions (including the concentration of substrates, the amount of lipase and, reaction temperature and reaction time). This might because the chemical modification of lipase via covalent immobilization, some active sites were covered up during immobilizing or absorption lipase on supports and the lipase tend to aggregate (Palomo, J.M., Fuentes, M., Fernández-Lorente, G., Mateo, C., Guisan, J. M., Fernández-Lafuente, R. *General trend of lipase to self-assemble giving bimolecular aggregates greatly modifies the enzyme functionality* (2003) Biomacromolecules, 4 (1), pp. 1-6.) in solution caused decrease of activity.

2. Effect of temperature on the activity of FCRL and C-ICRL

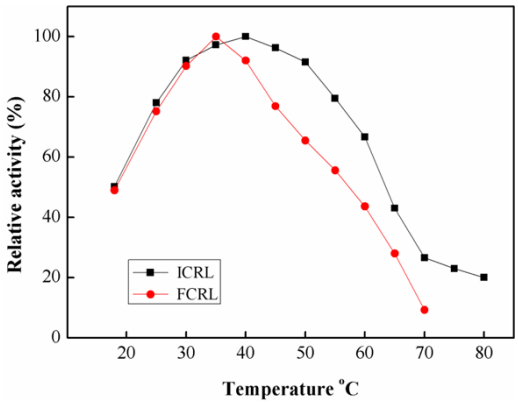

Figure s-2 Effect of temperature on the activity of FCRL and C-ICRL

Optimal conditions of enzymatic activity: effect of pH value and temperature on the enzymatic activity of C-ICRL. The above figure shows the effect of temperature on the activities of C-ICRL. As shown in figure s-2, the optimal enzymatic temperature of FCRL was 35 °C, while it was 40 °C for C-ICRL. Also, C-ICRL displayed good enzymatic activity in the temperature range of 35 °C to 55 °C. This excellent performance of C-ICRL might because of the covalent connection between enzyme and supports, which could improve the stability of enzyme and prevented enzyme from conformation transition in harsh operational conditions.

3. Effect of pH value on the activity of FCRL and C-ICRL

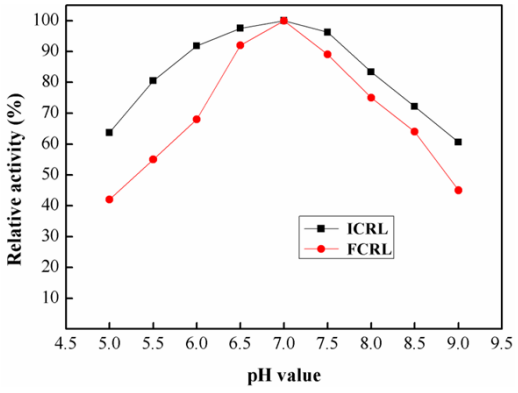

Figure s-3 effect of pH value on the activity of FCRL and ICRL

Figure s-3 shows the effect of pH value on the activity of C-ICRL. It can be observed that the optimal pH value of the activity of both ICRL and FCRL was 7.0, but C-ICRL acted out better pH tolerance than FCRL. In a wider pH range (5.5-8.0), ICRL expressed excellent enzymatic activity (relative activity > 80 %), while the activity of FCRL decreased rapidly when the pH values were away from 7.0. It might because a micro-environment provided by the nanoscale supports make C-ICRL a better tolerance of pH changes.

4. Reusability of ICRL

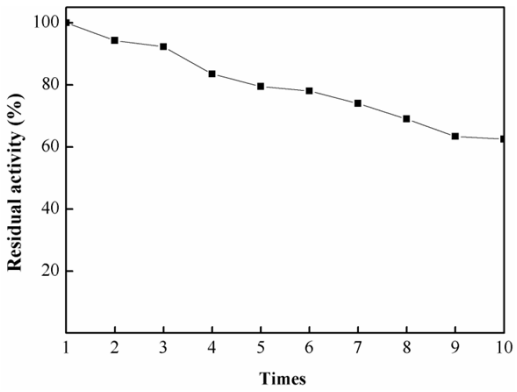

Figure s-4 (a) Reusability of C-ICRL

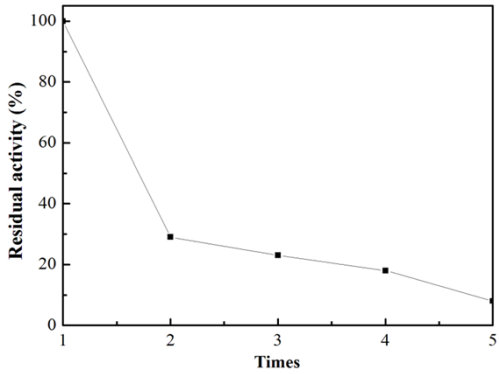

Figure s-4 (b) Reusability of I-ICRL

As shown in figure s-4, after being reused 10 times, ICRL with chemical and physical methods still showed good enzymatic activity (the relative activity > 60 %). In addition, CRL which was immobilized on the supports with only ionic exchange showed poor reusability after being reused 5 times (the relative activity < 10%).

5. Distribution range of the superparamagnetic Fe_3O_4 nanoparticles diameter

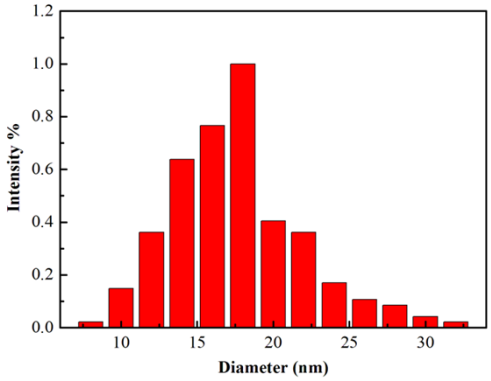

Figure s-5 Distribution range of the superparamagenetic Fe_3O_4 nanoparticles diameter

6. Loading yields

Table s-1(a) Enzyme loading of C-ICRL

Lipase amount added (mg/g)	50	100	150	200	250	300	350	400
Enzyme loading (mg/g)	37.76	80.3	129.2	174.6	221.3	268.8	315.0	356.8
Loading yield (%)	75.5	80.3	86.1	87.3	88.5	89.6	90.0	89.2

Table s-1(b) Enzyme loading of I-ICRL

Lipase amount added (mg/g)	10	20	30	40	50	60	70	80	90
Enzyme loading (mg/g)	0.8	2.8	5.4	8.8	11.1	19.4	33.3	46.1	57.8
Loading yield (%)	7.6	14.2	17.94	22.0	22.1	32.3	47.6	57.6	64.2

The effect of lipase amount added on the loading amount of ICRL was given in table s-1. It is clear that the loading amount of ICRL was enhanced as the lipase amount increased. The reason is believed to be that the Fe_3O_4 nanoparticles can provide a larger specific surface area and more active chemical site for immobilizing CRL. But the catalytic activity of C-ICRL reached maximum when the lipase amount added was 250 mg/g. And the optimal lipase amount added of I-ICRL was 80 mg/g within the scope of our research. The same phenomenon was reported in our team previously (see Xinghua Li and Hao Zhu's paper, *One-pot polylol synthesis of graphene decorated with size- and density-tunable Fe_3O_4 nanoparticles for porcine pancreatic lipase immobilization, carbon,* 2013, 60,488-497).

7. The SDS-PAGE of free CRL we used.

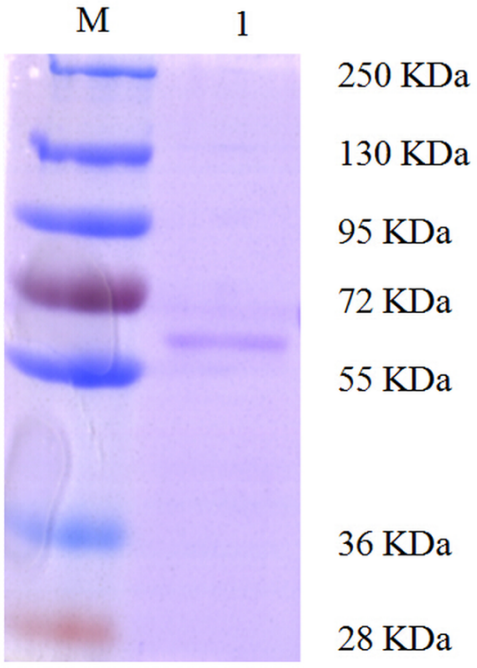

Figure s-6 The SDS-PAGE of free CRL we used. *Lane M* marker; *lane 1* free CRL.

It can be concluded that the free CRL was pure and the determination of the lipase immobilization via Bradford´s method is suitable.