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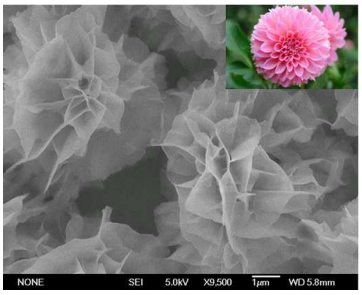

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PAPER

Enantioselective transesterification of (*R,S*)-2-pentanol catalyzed by a new flower-like nanobioreactor

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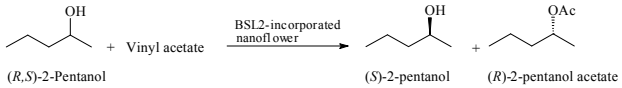
The lipase-incorporated nanoflower was prepared and used for resolution of (*R,S*)-2-pentanol with vinyl acetate as acyl donor in organic solvents. SEM images indicated that the lipase-incorporated nanoflower had high surface area which was favourable for the mass transfer. The FTIR spectrum identified the presence of the lipase in the nanoflower. The lipase-incorporated nanoflower could display its maximum enzyme activity (22.5 μmol h^{-1} mg^{-1}) and good enantioselectivity (*E* value, 47.3) under the optimal reaction conditions (in cyclohexane at 60 °C and water activity of 0.10). After ten continuous batches, the nanoflower remained 98.7% of initial enzyme activity and 95.6% of initial enantioselectivity.

Introduction

(*S*)-2-pentanol is a chiral intermediate for synthesizing several potential anti-Alzheimer's drugs that can suppress the formation and the release of β-amyloid peptide.[1] So far, many reports have focused on the preparation of (*S*)-2-pentanol with high enantiopurity. For instance, Iborra' group has reported that lipase B from *Candida antarctica* can present high enantioselectivity in the kinetic resolution of (*R,S*)-2-pentanol in the ionic liquid [Bmim][NTf_2].[2] Villora and Wang had also successfully carried out the enantioselective transesterification of (*R,S*)-2-pentanol catalyzed by lipase.[3-4] All these reports have used vinyl esters as acyl donor because the vinyl alcohol would be released from the vinyl esters during the transesterification and convert to acetaldehyde that make the reaction irreversible.[5-6] However, the generated acetaldehyde might attack the ε-amino of the lysine in the lipase to form Schiff's bases and decrease the enzyme activity.[6] Moreover, free lipase tended to aggregate together in the presence of a small amount of water in organic media that might also impair the enzyme performance. It's known that enzyme immobilization is one of the most efficient strategies for solving these problems.[7-12]

In 2012, Zare and his co-workers prepared a new hybrid organic-inorganic nanomaterial which consisted of copper phosphate and various enzymes.[13] Such hybrid organic-inorganic nanomaterial was formed *via* the coordination between Cu^{2+} and the nitrogen atoms of the amide groups in the enzymes. Because the shape of the hybrid organic-inorganic nanomaterial looked like a flower in nature, this hybrid nonmaterial was named nanoflower. The nanoflower has large surface-to-volume ratio, which is likely to have important applications in immobilization. In the present study, lipase-incorporated nanoflower was prepared for boosting the performance of lipase from B. *subtilis* (BSL2) in resolution of (*R,S*)-2-pentanol (Scheme 1) and the reaction conditions had been optimized. Furthermore, the reusability of the BSL2-incorporated nanoflower had also been studied.



Scheme 1: Resolution of (*R,S*)-2-pentanol catalyzed by BSL2-incorporated nanoflower using vinyl acetate as acyl donor.

Experiment

Materials

Bacillus subtilis lipase (BSL2, 24 kDa) was prepared in our laboratory.[14-15] (*R,S*)-2-pentanol and vinyl acetate were purchased from Sigma-Aldrich-Fluka Chemical Co (St. Louis, MO, USA) and were of the highest purity available. KBr (spectral grade) was obtained from BDH Co. (Poole, UK). All other chemicals and reagents were of analytical grade. All aqueous solutions were prepared with Milli-Q water.

Preparation of BSL2

The B. *subtilis* strain BSL2 was constructed by Dr. Ma J. S. in our laboratory.[16] A culture medium (LB), comprising of 1.0% tryptone, 0.5% yeast extract and 1.0% sodium chloride (NaCl) (pH 7.0 before sterilization and pH 6.8 after sterilization) was prepared and dispensed in shake flasks (100 ml and 5 l, respectively). The preculture of B. *subtilis* strain BSL2 was made by shaking the cells in LB supplemented with 30 mg/l kanamycin and incubating the flask at 30 °C on a rotary shaker. The 28 h old preculture thus produced was inoculated (1%, v/v) into 1 l of LB with 30 mg/l kanamycin, and fermentation was carried out at 500 rpm keeping at a constant temperature of 30 °C for 28–30 h. The culture was thereafter centrifuged to collect the cells. The cell pellet was washed twice with 30 mmol/l phosphate buffer (pH 7.0) and the wet cell mass (5.8 g) was thus obtained. The wet cell mass was crushed by sonication and the supernatant was obtained by centrifugation at 12,000 rpm for 30 min at 4 °C. The crude enzyme was partially purified by ammonium sulfate fractionation procedure. The ammonium sulfate precipitation was dialyzed in 30 mmol/l phosphate buffer (pH 7.0) to remove the excess salt.

The enzyme was used after lyophilization.

Preparation of BSL2-incorporated nanoflower

The synthesis of BSL2-incorporated nanoflower was carried out according to the previous work with a slight modification.[13] At first, 60 ml of BSL2 solution (1 mg/ml) was added to 3 l of PBS solution (50 mmol/l, pH 7.4), followed by the addition of 20 ml of $CuSO_4$ solution (120 mmol/l). Then, the mixture was incubated at 25 °C for three days. The blue product could be found at the bottom of the flash. Finally, the blue product was collected by centrifugation (12,000 rpm for 20 min) and washed by deionized water for three times. The protein concentration in the supernatant was quantified using the Branford protein assay with BSA as a standard.[17] As a result, the immobilization yield of protein was determined. Since no protein in the pooled supernatant and washing solutions was detected, the immobilization yield of BSL2 was nearly 100%.

Characterization of BSL2-incorporated nanoflower

The morphologies of the samples were observed by a JSM-6700F electron microscope (JEOL, Japan) with an acceleration voltage of 30 kV. The FTIR spectrums of the samples were surveyed using Nicolet 5700 FTIR spectrometer with a resolution of 4 cm^{-1} through KBr method.

Resolution of (*R,S*)-2-pentanol catalyzed by BSL2-incorporated nanoflower

The reaction system containing (*R,S*)-2-pentanol (1 mmol), vinyl acetate (2.5 mmol), cyclohexane (5 ml), water activity (a_w=0.10) and BSL2-incorporated nanoflower (10 mg) was incubated at 60 °C.

Water activity (aw) control

The water activity was controlled by Tian's method.[18] All reaction mixtures underwent the dry treatment in a vacuum of 1 mmHg for 12 h. Then, all reaction mixtures with a given water activity (a_w) were obtained by adding a given amount of water. Then, equilibration was implemented at room temperature for 24 h in a sealed vial. Water activity (a_w) was determined by Hygrolab Humidity Detector (Rotronic, Switzerland).

Reusability

BSL2-incorporated nanoflower was used for successive batches. After each cycle, reaction mixture was centrifuged at 12,000 rpm for 20 min. The obtained precipitate was washed with cyclohexane for three times to remove any residual substrate or product and then kept overnight in a vacuum oven for a complete drying. Dried nanoflower was used at next cycle. The enzyme activity and the *E* value of the samples were measured as described below.

Determination of enantiomeric excess and *E* value

GC analysis was carried out using an Agilent 6890N instrument equipped with flame ionization detector (FID) and an HP-chiral (30 m × 0.25 mm) chiral capillary column. The injector and the detector were kept at 250 °C, and the column temperature was programmed to maintain 65 °C for 40 min. Nitrogen was employed as the carrier gas with a constant flow rate (1.8 ml min^{-1}). The split flow was 40 ml min^{-1}. The enzyme activity (μmol h^{-1} mg^{-1}) was defined as the amount of 2-pentanol acetate produced per hour per milligram of the immobilized enzyme. The *E* value was calculated at a certain conversion (*c*, %) through the following equation.[19]

$$ee_s(\%) = \frac{[S-R]}{[S+R]} \times 100 \quad (1)$$

$$E \text{ value } E = \frac{\ln[(1-c)(1-ee_s)]}{\ln[(1-c)(1+ee_s)]} \quad (2)$$

where *S* and *R* represent the concentrations of the (*S*)-2-pentanol and (*R*)-2-pentanol, respectively.

Results and discussion

SEM

The SEM images showed that a large number of BSL2-incorporated nanoflowers could be successfully prepared (Fig. 1A and inset of Fig. 1A). All samples exhibited flower-shape with a diameter of 4–5 μm (Fig. 1B and Fig. 1C) and the morphology of nanoflower directed by BSL2 was similar to the shape of dahlia in nature (Inset of Fig. 1C) that possessed dozens of separate petals with high surface (Fig. 1C). It could also be found from Fig. 1D that there is no formation of nanoflower in the absence of BSL2. Therefore, it is believed that BSL2 acts as not only the capping agents to modulate the growth of the nanoflower but also the biotemplate to direct the formation of the hierarchical structure of the nanoflower.[20]

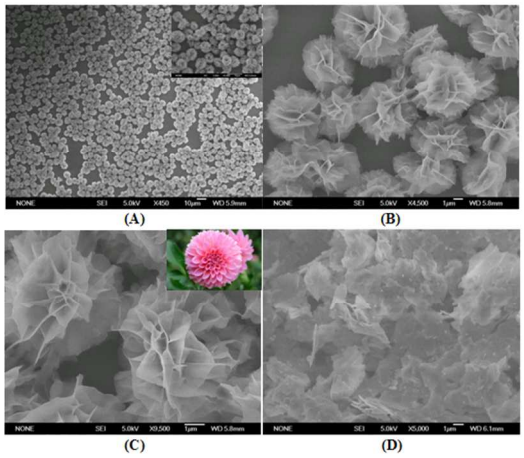


Fig. 1 SEM of the samples in the presence or absence of BSL2. A-C and inset of A: BSL2-incorporated nanoflowers with different enlargement factors. Inset of C: The image of dahlia in nature. D: $Cu_3(PO_4)_2.3H_2O$ matrices without BSL2.

FTIR

The FTIR spectrums of the $Cu_3(PO_4)_2.3H_2O$ matrices without BSL2, BSL2 and BSL2-incorporated nanoflower were shown in Fig. 2. The peaks at 1053 cm^{-1} and 556 cm^{-1} are assigned to the vibrations of PO_4^{3-} (curve 1 and curve 3 in Fig. 2).[21] The peaks at 1658 cm^{-1} and 1536 cm^{-1} are ascribed to the vibrations of the amide I and II bands of BSL2 (curve 2 and curve 3 in Fig. 2).[22] The amide I band at 1658 cm^{-1} is mainly attributed to the contribution of C=O stretching vibrations of the amide groups in BSL2. The amide II at 1536 cm^{-1} derives mainly from in-plane NH bending vibration and CN stretching vibration in BSL2.[23]

These results verified the presence of BSL2 in the nanoflower.

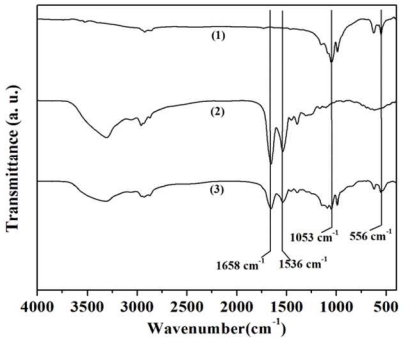


Fig. 2 The FTIR spectrum of $Cu_3(PO_4)_2.3H_2O$ matrices without BSL2 (curve 1), BSL2 (curve 2) and BSL2-incorporated nanoflower (curve 3)

The effect of organic solvent

The prepared nanoflower was used as bioreactor to catalyze the resolution of (*R,S*)-2-pentanol. Generally speaking, the solvent has a profound effect on the enzyme performance in non-aqueous enzymology.[24-26] A survey involving seven solvents with different log *P* was implemented in this work to determine the optimal reaction medium for resolution of (*R,S*)-2-pentanol. The results shown in Table 1 indicated that the best enzyme activity and the *E* value of BSL2-incorporated nanoflower were obtained when cyclohexane was used as the solvent.

Table 1 The effect of organic solvent on BSL2-incorporated nanoflower [a]

	log *P*	Enzyme activity (μmol h^{-1} mg^{-1})	*E* value	Stereoselectivity
n-Hexane	3.50	20.9 ± 1.0	45.5 ± 3.0	*R*
Cyclohexane	3.20	22.5 ± 0.8	47.3 ± 3.0	*R*
Toluene	2.50	19.0 ± 1.0	39.4 ± 2.8	*R*
Acetone	-0.23	14.7 ± 1.0	37.5 ± 3.0	*R*
Acetonitrile	-0.33	8.3 ± 0.4	27.6 ± 2.8	*R*
DMF	-1.00	5.3 ± 0.4	17.4 ± 2.6	*R*
1,4-Dioxane	-1.10	2.3 ± 0.2	8.0 ± 0.5	*R*

[a] Reaction conditions: (*R,S*)-2-pentanol (1 mmol), vinyl acetate (2.5 mmol), water activity (a_w=0.10), BSL2-incorporated nanoflower (10 mg) and different organic mediums (5 ml) at 60 °C.

The effect of temperature

It's known that the temperature can influence the catalytic efficiency of the used enzyme.[27] In this study, the effect of temperature on the performance of BSL2-incorporated nanoflower in resolution was investigated in the range of 35-70 °C. As shown in Fig. 3, the enzyme activity of BSL2 in the nanoflower increased with the increasing temperature ranging from 35 °C to 60 °C, and then decreasing with the further increase of temperature (60-70 °C). The maximum enzyme activity was observed at 60 °C. As for the enantioselectivity, the *E* value of BSL2-incorporated nanoflower gradually decreased with the increasing temperature. It could be found that low temperature (<60 °C) could increase the enantioselectivity owing to the enthalpic control, but decrease the enzyme activity.[28-29] So, the choice of optimal temperature in an enzymatic kinetic resolution always is a compromise between the enzyme activity and the enantioselectivity. In this study, 60 °C was chosen as the optimal reaction temperature for the following experiments.

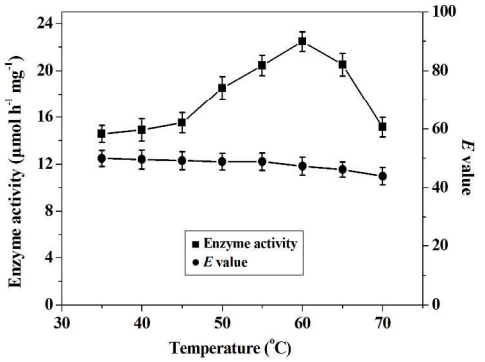


Fig. 3 The effect of temperature on the enzyme activity (■) and the *E* value (●) of BSL2-incorporated nanoflower. Reaction conditions: (*R,S*)-2-pentanol (1 mmol), vinyl acetate (2.5 mmol), cyclohexane (5 ml), water activity (a_w=0.10) and BSL2-incorporated nanoflower (10 mg) at different temperatures (35-70 °C).

The effect of water activity (a_w)

To clarify the influence of water activity on the performance of BSL2-incorporated nanoflower, the enzyme activity and the *E* value of BSL2 in the nanoflower were measured at different water activities (0.02-0.50). As shown in Fig. 4, the enzyme activity exhibited a bell-shaped curve and the maximum value was obtained at a_w 0.10. Furthermore, it could also be found that the *E* value of BSL2-incorporated nanoflower kept almost constant at various water activities (0.02-0.50), which was in agreement with our previous reports.[9, 30] The highest enzyme activity at the optimal water activity (a_w, 0.10) might be due to the lubricant effect of water molecules.[31-32]

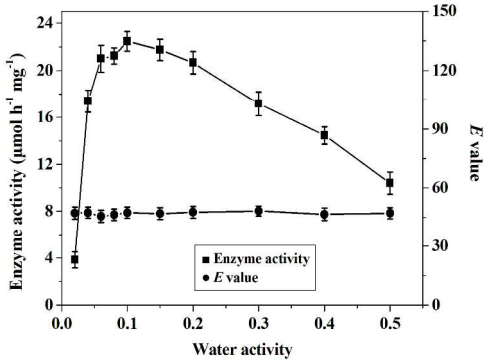


Fig. 4 The effect of water activity on the enzyme activity (■) and the *E* value (●) of BSL2-incorporated nanoflower. Reaction conditions: (*R,S*)-2-pentanol (1 mmol), vinyl acetate (2.5 mmol), cyclohexane (5 ml) and BSL2-incorporated nanoflower (10 mg) at 60 °C at different water activities (a_w 0.02-0.50).

Reusability

In practical application, the reusability of the enzyme is of considerable importance and the immobilization can facilitate the enzyme recovery.[33-34] For checking the parameter, BSL2-incorporated nanoflower was implemented in five continuous

batches for resolution of (*R,S*)-2-pentanol under the optimal reaction conditions. After ten cycles, BSL2 in the nanoflower maintained 98.7% of initial enzyme activity and 95.6% of initial *E* value (Table 2). The loss of the enzyme activity and enantioselectivity of BSL2-incorporated nanoflower was almost negligible. The results indicated that BSL2 couldn't escape from the nanoflower and exhibited an excellent reusability.

Table 2 Reusability of BSL2-incorporated nanoflower [a]

Batch	Enzyme activity ($\mu mol\ h^{-1}\ mg^{-1}$)	*E* value
1	22.5 ± 0.8	47.3 ± 3.0
2	22.5 ± 0.9	47.2 ± 2.7
3	22.4 ± 0.8	47.0 ± 2.5
4	22.4 ± 0.6	46.8 ± 3.1
5	22.4 ± 0.9	46.1 ± 2.9
6	22.3 ± 0.7	45.8 ± 3.0
7	22.3 ± 0.8	45.7 ± 2.6
8	22.3 ± 0.9	45.5 ± 2.9
9	22.2 ± 0.6	45.3 ± 2.7
10	22.2 ± 0.8	45.2 ± 3.0

[a] Reaction conditions: (*R,S*)-2-pentanol (1 mmol), vinyl acetate (2.5 mmol), cyclohexane (5 ml), water activity (a_w=0.10) and BSL2-incorporated nanoflower (10 mg) at 60 °C.

Resolution of other chiral alcohols

The BSL2-incorporated nanoflower had also been used for resolution of several other chiral alcohols. All the reactions were under their optimal reaction conditions which were listed below the Table 3. It could be found that all the reactions could be carried out with satisfied results and the nanoflower exhibited better enzyme performance than the free enzyme. The improved performance of the BSL2-incorporated nanoflower probably arises from the following conjectures: (I) Nanoflower protects BSL2 from the attack of acetaldehyde. (II) The aggregations between BSL2 molecules are well inhibited after being entrapped into the nanoflower.

Table 3 Resolution of other chiral alcohols catalyzed by BSL2-incorporated nanoflower

Substrates	Enzyme activity ($\mu mol\ h^{-1}\ mg^{-1}$)		*E* value	
	Free BSL2	Nanoflower	Free BSL2	Nanoflower
2-Pentanol [a]	12.6 ± 0.7	22.5 ± 0.8	33.9 ± 2.9	47.3 ± 3.0
2-Octanol [b]	21.7 ± 0.3	35.8 ± 0.5	72.4 ± 2.7	83.8 ± 3.2
Glycidol [c]	42.5 ± 0.4	78.7 ± 0.7	30.4 ± 2.6	41.5 ± 2.7
1-Phenylethanol [d]	2.1.± 0.3	3.3 ± 0.5	17.6 ± 3.1	32.2 ± 2.5
2-Methyl-1-butanol [e]	15.2 ± 0.7	38.9 ± 0.8	21.5 ± 2.7	25.6 ± 2.9

[a] Reaction conditions: (*R,S*)-2-pentanol (1 mmol), vinyl acetate (2.5 mmol), cyclohexane (5 ml), water activity (a_w=0.10) and BSL2-incorporated nanoflower (10 mg) / free BSL2 (2 mg) at 60 °C.

[b] Reaction conditions: (*R,S*)-2-octanol (0.5 mmol), vinyl acetate (1 mmol), *n*-hexane (5 ml), water activity (a_w=0.45) and BSL2-incorporated nanoflower (10 mg) / free BSL2 (2 mg) at 50 °C.

[c] Reaction conditions: (*R,S*)-glycidol (5 mmol), vinyl butyrate (15 mmol), 1,2-dichloroethane (5 ml), water activity (a_w =0.33) and BSL2-incorporated nanoflower (10 mg) / free BSL2 (2 mg) at 40 °C.

[d] Reaction conditions: (*R,S*)-1-phenylethanol (0.5 mmol), vinyl butyrate (1 mmol), cyclohexane (5 ml), water activity (a_w =0.53) and BSL2-incorporated nanoflower (10 mg) / free BSL2 (2 mg) at 60 °C.

[e] Reaction conditions: (*R,S*)-2-methyl-1-butanol (1 mmol), vinyl acetate (4 mmol), cyclohexane (5 ml), water activity (a_w =0.51) and BSL2-incorporated nanoflower (10 mg) / free BSL2 (2 mg) at 45 °C.

Conclusion

In present work, the lipase-incorporated nanoflower was prepared and successfully used for resolution of (*R,S*)-2-pentanol with vinyl acetate as acyl donor in cyclohexane (a_w, 0.10) at 60 °C. Our results indicated that the lipase-incorporated nanoflower could present excellent enzyme activity and enantioselectivity in the reaction. Furthermore, the new flower-like nanobioreactor exhibited excellent reusability. In future, more work about the nanoflower will be done to clarify its formation mechanism and widen its application in various fields.

Acknowledgments

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Notes and references

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1. R. N. Patel, *Adv. Synth. Catal.*, 2001, 343, 527-546.
2. M. Noël, P. Lozano, M. Vaultier and J. L. Iborra, *Biotechnol. Lett.*, 2004, 26, 301-306.
3. F. Hernández-Fernández, A. de Los Rios, F. Tomás-Alonso, D. Gómez and G. Víllora, *J. Membrane Sci.*, 2008, 314, 238-246.
4. Y. Wang, Q. Li, Z. Zhang, J. Ma and Y. Feng, *J. Mol. Catal. B: Enzym.*, 2009, 56, 146-150.
5. T. M. Donohue Jr, D. J. Tuma and M. F. Sorrell, *Arch. Biochem. Biophys.*, 1983, 220, 239-246.
6. H. Kaga, B. Siegmund, E. Neufellner, K. Faber and F. Paltauf, *Biotechnol. Tech.*, 1994, 8, 369-374.
7. B. Berger and K. Faber, *J. Chem. Soc., Chem. Commun.*, 1991, 1198-1200.
8. E.-n. Xun, X.-l. Lv, W. Kang, J.-x. Wang, H. Zhang, L. Wang and Z. Wang, *Appl. Biochem. Biotechnol.*, 2012, 168, 697-707.
9. C. Du, B. Zhao, C. Li, P. Wang, Z. Wang, J. Tang and L. Wang, *Biocatal. Biotransform.*, 2009, 27, 340-347.
10. M. C. Franssen, P. Steunenberg, E. L. Scott, H. Zuilhof and J. P. Sanders, *Chem. Soc. Rev.*, 2013, 42, 6491-6533.
11. R. A. Sheldon and S. van Pelt, *Chem. Soc. Rev.*, 2013, 42, 6223-6235.
12. Y. Masuda, S.-i. Kugimiya, Y. Kawachi and K. Kato, *RSC Adv.*, 2014, 4, 3573-3580.
13. J. Ge, J. Lei and R. N. Zare, *Nat. Nanotechnol.*, 2012, 7, 428-432.

14. L. Wang, J. Tai, R. Wang, E. Xun, X. Wei, L. Wang and Z. Wang, *Biotechnol. Appl. Biochem.*, 2010, 56, 1-6.
15. C. Li, P. Wang, D. Zhao, Y. Cheng, L. Wang, L. Wang and Z. Wang, *J. Mol. Catal. B: Enzym.*, 2008, 55, 152-156.
16. J. Ma, Z. Zhang, B. Wang, X. Kong, Y. Wang, S. Cao and Y. Feng, *Protein Expr. Purif.*, 2006, 45, 22-29.
17. M. M. Bradford, *Anal. Biochem.*, 1976, 72, 248-254.
18. R. Tian, C.-H. Yang, X.-F. Wei, E.-N. Xun, R. Wang, S.-G. Cao, Z. Wang and L. Wang, *Biotechnol. Bioprocess Eng.*, 2011, 16, 337-342.
19. C. S. Chen, Y. Fujimoto, G. Girdaukas and C. J. Sih, *J. Am. Chem. Soc.*, 1982, 104, 7294-7299.
20. J. Zeng and Y. Xia, *Nat. Nano.*, 2012, 7, 415-416.
21. I. S. Cho, D. W. Kim, S. Lee, C. H. Kwak, S. T. Bae, J. H. Noh, S. H. Yoon, H. S. Jung, D. W. Kim and K. S. Hong, *Adv. Funct. Mater.*, 2008, 18, 2154-2162.
22. W.-J. Yang, P. R. Griffiths, D. M. Byler and H. Susi, *Appl. Spectrosc.*, 1985, 39, 282-287.
23. S. KR, Adv. *Protein Chem.*, 1986, 38, 181.
24. K. Ryu and J. S. Dordick, *Biochemistry*, 1992, 31, 2588-2598.
25. C. Laane, S. Boeren, K. Vos and C. Veeger, *Biotechnol. Bioeng.*, 1987, 30, 81-87.
26. R. Wedberg, J. Abildskov and G. n. H. Peters, *J. Phys. Chem. B*, 2012, 116, 2575-2585.
27. P. A. Overbeeke, J. Ottosson, K. Hult, J. A. Jongejan and J. A. Duine, *Biocatal. Biotransform.*, 1999, 17, 61-79.
28. R. S. Phillips, *Trends Biotechnol.*, 1996, 14, 13-16.
29. V. Léonard, L. Fransson, S. Lamare, K. Hult and M. Graber, *ChemBioChem*, 2007, 8, 662-667.
30. W. Lei, T. Jian-Dong, W. Ren, X. Er-Na, W. Xiao-fei and W. Zhi, *Biotechnol. Appl. Biochem.*, 2010, 56, 1-6.
31. A. Zaks and A. M. Klibanov, *J. Biol. Chem.*, 1988, 263, 8017-8021.
32. P. Adlercreutz, *Chem. Soc. Rev.*, 2013, 42, 6406-6436.
33. U. Hanefeld, L. Gardossi and E. Magner, *Chem. Soc. Rev.*, 2009, 38, 453-468.
34. Z. Wu, Z. Wang, B. Guan, X. Wang, Y. Zhang, Y. Xiao, B. Zhi, Y. Liu, Z. Li and Q. Huo, *New J. Chem.*, 2013, 37, 3793-3797.