# Carboligation reactions with benzaldehyde lyase immobilized on superparamagnetic solid support

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Histidine-tagged recombinant benzaldehyde lyase (BAL, EC 4.1.2.38) was efficiently immobilized to surface-modified magnetic particles with affinity ligand binding. In addition to conventional benzoin condensation reactions, two important representative BAL-catalyzed carboligation reactions, were also performed with this magnetically responsive biocatalyst. The results obtained from the carboligation reactions that were performed with this simple and convenient heterogenous biocatalyst were comparable to that of free-enzyme-catalyzed reactions.

## Introduction

Benzaldehyde lyase (BAL, EC 4.1.2.38), a thiamine pyrophosphate (TPP) dependent enzyme, is a rather important biocatalyst for enantioselective carboligation reactions.[1] The enzyme can produce chiral α-hydroxyketones that are indispensable building blocks in the synthesis of several compounds. Many novel reactions of the native and recombinant enzyme have been reported, in which the applicability of BAL has already been extended through a wide range of substrate spectra.[2]

Immobilization is one of the most efficient methods for increasing the cost effectiveness of enzymatic reactions.[3] The catalyst can be stabilized and reused by entrapment on different materials. Functionalized magnetic particles have been extensively used in the immobilization of many bioactive substances, such as proteins, peptides, enzymes, and antibodies.[4] Magnetic particles can be easily recovered from media by applying a magnetic field. When they are used for enzyme immobilization, particularly in batch reactors and continuous-flow stirred-tank reactors (CSTR), this method facilitates the separation of enzymes from the product after the completion of the reaction in order to permit the reuse and recycling of the enzyme.[5] The synthesis of nitrilotriacetic acid (NTA) coated magnetic nanoparticles has been reported for enzyme immobilization applications.[6] These micron- or nano-scale particles selectively bind to histidine-tagged recombinant proteins and combine the advantages of magnetically responsive particles and the metal affinity ligand within enzyme purification and immobilization studies.

Carboligation reactions that are catalyzed by immobilized BAL have been reported.[7,8] In addition to an increase in the cost effectiveness of BAL-catalyzed reactions, the design of an immobilized biocatalyst for continuous processes was aimed at,[7] as well as to stabilize the enzyme in order to be used in organic media.[8] Since recombinant histidine tagged BAL can be produced by overexpressing a recombinant *E. coli* strain, it is possible to make use of metal affinity ligands within immobilization studies. Herein, we aimed at the introduction of a heterogeneous biocatalyst that offers the ease of immobilization along with the separation steps of metal affinity ligand ($Co^{2+}$-NTA) coated magnetic particles. These methods are also well known for recombinant protein purification, in which our surface modified magnetic system could also be used for the preparation of isolated BAL for free enzyme catalyzed carboligation reactions. For the abovementioned purposes, we synthesized superparamagnetic γ-$Fe_2O_3$ (maghemite)–silica nanocomposite particles by using the sol–gel method, in which the surface modification method was applied for the ease of the purification of histidine tagged proteins.[9] A magnetically responsive biocatalyst system was prepared by affinity adsorption of BAL, and self condensation reaction of benzaldehyde **1** was performed in this convenient batch catalyst system. In addition to this conventional benzoin condensation reaction, a magnetic resin–BAL biocatalyst was also tested for two important representative reactions—kinetic resolutions of racemic benzoin **2** with the lyase activity of the BAL in turn resulting in cross condensation with acetaldehyde[10] and the synthesis of (*R*)-2-hydroxy-3,3-dimethoxy-1-(4-methoxyphenyl)propan-1-one (**5**), which is a cross condensation product of *p*-anisaldehyde (**4**) and dimethoxy acetaldehyde.[11]

## Results and discussion

### Characterization of surface modified γ-$Fe_2O_3$–silica nanocomposite particles

We prepared surface modified maghetite particles by using the optimized conditions that were described by Tural *et al.* previously.[9] γ-$Fe_2O_3$ (maghemite)–silica nanocomposite particles were synthesized by using the sol–gel method. The condensation products of 3-glycidoxypropyltrimethoxysilane (GPTMS) and *N*α,*N*α-bis(carboxymethyl)-L-lysine hydrate (NTA) were introduced onto the surfaces of the γ-$Fe_2O_3$–silica nanocomposite particles and, subsequently, these modified surfaces were complexed with cobalt ($Co^{2+}$) metal ions. The schematic illustration for the preparation steps of $Co^{2+}$ charged γ-$Fe_2O_3$–silica-nanocomposite particles that were used for benzaldehyde lyase adsorption is shown in Scheme 1.

The magnetization curve at 300 K for the surface modified γ-$Fe_2O_3$–silica nanocomposite particles that were prepared by using an S/V ratio of 0.04 is shown in Fig. 1. The magnetization

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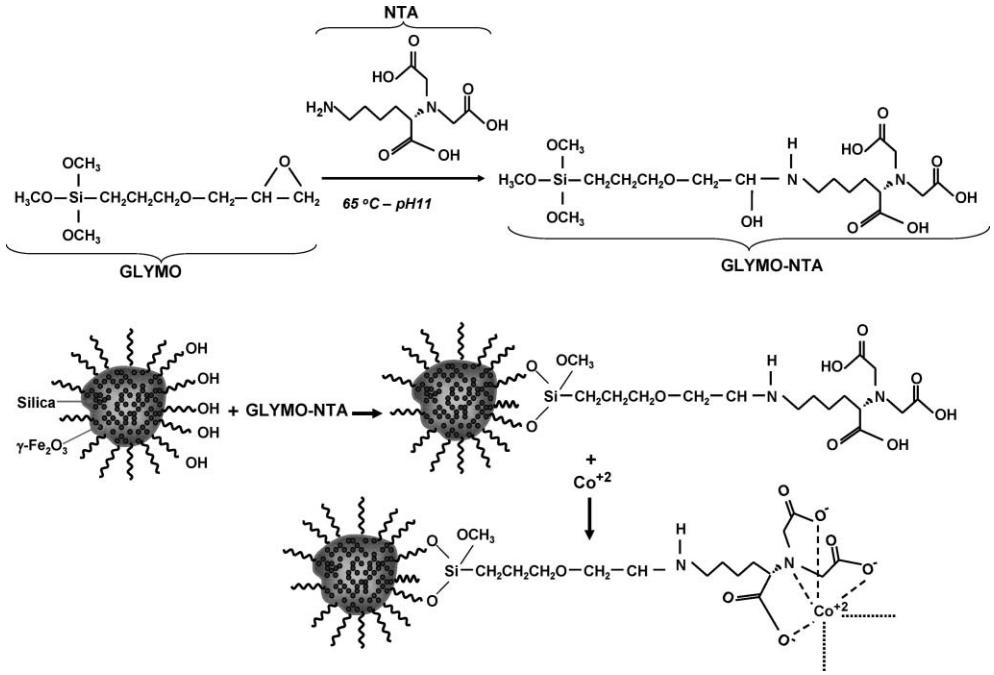



**Scheme 1** Schematic illustration for the preparation steps of $Co^{2+}$ charged $\gamma$-$Fe_2O_3$–silica nanocomposite particles used for benzaldehyde lyase adsorption.

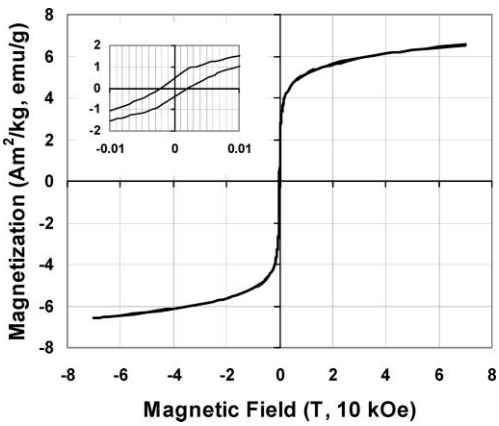



**Fig. 1** Magnetic hysteresis loops measured at 300 K for the surface modified $\gamma$-$Fe_2O_3$–silica nanocomposite particles prepared at the S/V ratio of 0.04.

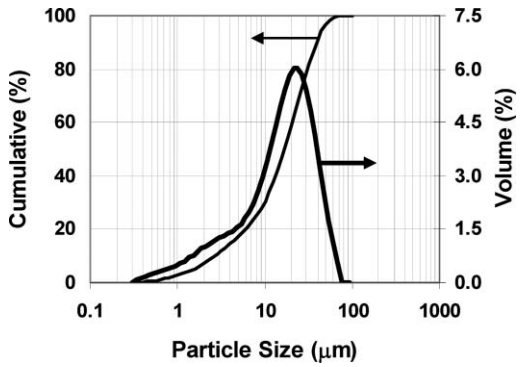



**Fig. 2** Particle size distribution for the surface modified $\gamma$-$Fe_2O_3$–silica nanocomposite particles.

curve of the surface modified $\gamma$-$Fe_2O_3$–silica nanocomposite particles nearly exhibit superparamagnetic behavior, showing slight hysteresis with the remanent magnetization and coercivity values of approximately 0.45 emu $g^{-1}$ and 20 Oe, respectively (see the inset in Fig. 2). The saturation magnetization values of the surface modified $\gamma$-$Fe_2O_3$–silica nanocomposite particles at 300 K were determined as 6.54 emu $g^{-1}$.

The particle size distribution for the surface modified $\gamma$-$Fe_2O_3$-silica nanocomposite particles are shown in Fig. 2. From the particle size distribution data, the volume weighted mean diameter (De Brouckere mean diameter, $D$[4,3]) and the surface area weighted mean diameter (Sauter mean diameter, D[3,2]) of the surface modified $\gamma$-$Fe_2O_3$–silica nanocomposite particles were determined as 19.2 μm and 6.3 μm, respectively. The specific surface area (*i.e.* the surface area per unit volume or unit mass of particles) of the $\gamma$-$Fe_2O_3$–silica nanocomposite particles, which can be calculated from the $D$[3,2] (specific surface area = $6/D$[3,2]), was determined as 0.95 $m^2$ $cm^{-3}$.

## Immobilization of BAL

First, $Co^{2+}$-NTA functionalized $\gamma$-$Fe_2O_3$ nanoparticles were employed for the one-pot purification–immobilization steps. It was shown by SDS-page analysis that our magnetic nanoparticles were efficient for the selective binding of HIS-tagged BAL (Fig. 3). The cell free extract of *E. coli* BL21(DE3)pLysS containing HIS-tagged BAL was incubated with resin, in which, after excessive washing, the protein was eluted and then the quantification of the protein was subsequently performed by a standard BSA protein assay.[12] The maximum protein amount that was attached to the resin was estimated as 3.16 mg $g^{-1}$.

The enzyme–magnetic resin biocatalyst system was highly responsive to a magnetic field, where the slurry was clarified in 30 s by using a regular magnet (Fig. 4a). Fig. 4b shows the TEM

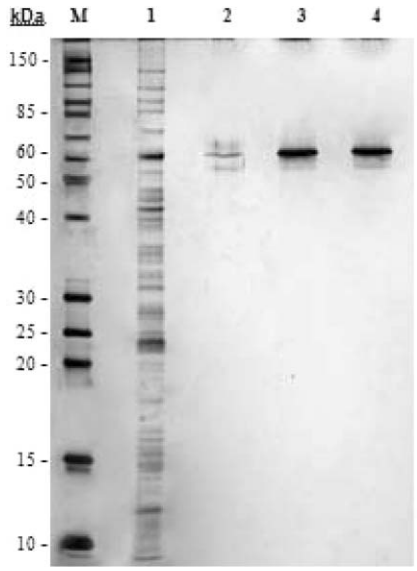



**Fig. 3** SDS-PAGE analysis of the purified 6Xhis tagged BAL. M, SDS–PAGE molecular weight; lane 1, crude extract of *E. coli* BL21(DE3)pLysS containing $BAL_{HIS}$; lane 2–4, protein eluted from 100, 700, 1500 µl crude extract loaded resin (50 µL sample from eluents).

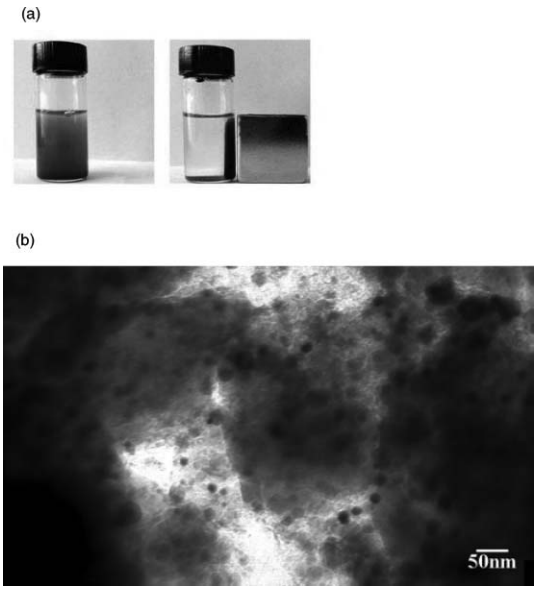
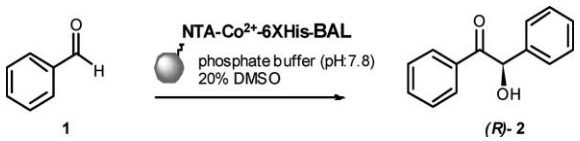



**Fig. 4** (a) Response of the BAL-magnetic resin biocatalyst to the magnetic field. (b) TEM bright field micrographs for the samples prepared at S/V ratio of 0.04 and gelated at 50 °C.[8]

bright field micrographs for the samples that were prepared at an S/V ratio of 0.04 and gelated at 50 °C.

### Benzoin condensation reaction

The benzoin condensation reaction of immobilized BAL was conducted in a batch synthesis (Scheme 2). The reaction conditions were chosen, as previously reported by Drager *et al.*[7], and kinetic analysis was performed by following an increase in the benzoin concentration (Fig. 5). The reaction curves of 5, 20 and 100 mg benzaldehyde (**1**) reached a plateau after 20 min, in which the conversion of benzaldehyde to (*R*)-benzoin (**2**) was low. Catalytic




**Scheme 2** BAL mediated self-condensation reaction of benzaldehyde.

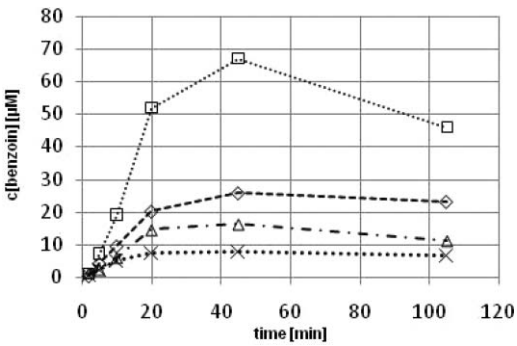



**Fig. 5** Time course of the change in the benzoin concentration during the reaction of BAL immobilized on $\gamma$-$Fe_2O_3$ nanoparticles at a different benzaldehyde concentration (△: 1 mg $mL^{-1}$, ◇: 4 mg $mL^{-1}$, □: 10 mg $mL^{-1}$, ×: 50 mg $mL^{-1}$).

activities were calculated from the initial reaction rates as 0.5 U, 0.9 U and 1.5 U. There was no activity detected with 200 mg $mL^{-1}$ benzaldehyde. However, the reaction conducted with 50 mg benzaldehyde resulted in a 90% conversion after 45 min as a result of the higher catalytic activity of this reaction (1.5 U), in which a decline in the benzoin concentration occurred at subsequent time points due to product inhibition. In all the cases, the reactions were followed up to 200 min, in which a fluctuation in the benzoin concentration was observed at a high concentration of benzaldehyde (100 mg). A similar observation was also reported by Drager *et al.*[7] with a benzaldehyde concentration during a BAL immobilized batch reaction. The lyase activity of BAL is reported as threefold lower[13] and, therefore, this fluctuation may also be explained by the cleavage of benzoin when the benzoin concentration reached saturation concentrations for the lyase reaction. It was also noted that benzaldehyde was less soluble at high concentrations (100 mg and 200 mg) and partially remains as small droplets in the reaction media for a longer time. This may explains the little-or-no activity at these higher benzaldehyde concentrations. As was reported by Pohl *et al.*,[13] during their kinetic analysis of BAL with free enzyme at a higher concentration of benzaldehyde, activity was increased when the co-solvent (DMSO) increased to 30%. The activity of immobilized BAL under optimized conditions was found to be comparable to that of free enzyme and enzyme immobilized to $Co^{2+}$-NTA TALON resin in a volume that contained an equal amount of protein with nanoparticles.

Repeated experiments were performed with the same batch of immobilized enzyme after washing with 50% DMSO in a lysis buffer. The resin was able to be reused without any loss of activity for the three sets of experiments, in which the respective activity decreased during the 4$^{th}$ repetition and the remaining activity was calculated as 96 and 86.5% for the 4$^{th}$ and 5$^{th}$ repetition, respectively (Fig. 6).

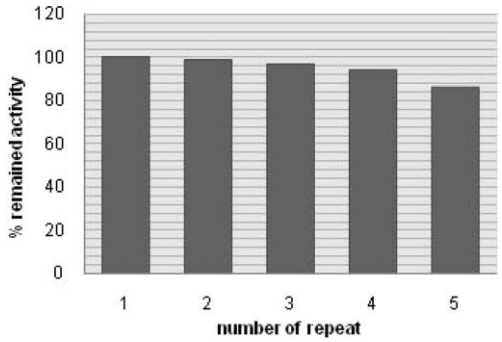



**Fig. 6** Remaining activity of the immobilized BAL with a repeated set of experiments.

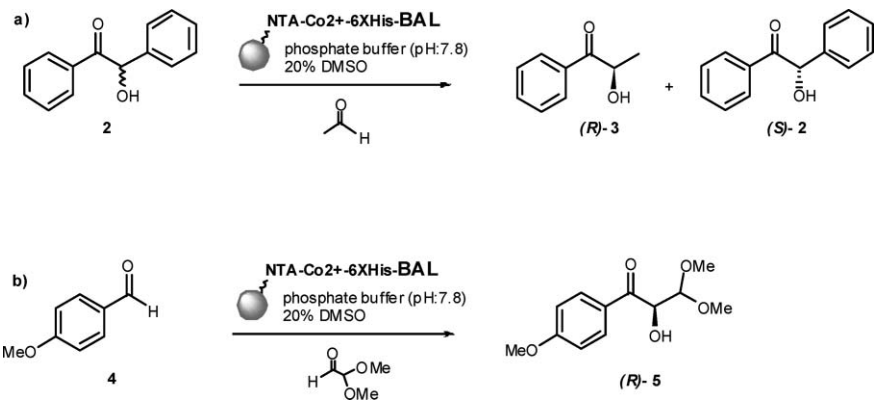
### Synthesis of HPP derivatives

In our previous work, we achieved the BAL mediated synthesis of (*R*)-2-hydroxy-1-phenylpropanone [(*R*)-2 HPP][9] (*R*)-**3** (starting from racemic benzoin and acetaldehyde) and 2-hydroxy-3,3-dimethoxy-1-(4-methoxyphenyl)propan-1-one (**5**).[10] In the former case, the reaction was first performed by lyase activity, which resulted in the kinetic resolution of racemic benzoin and ligase activity in turn promoting both cross and self condensation products (Scheme 3a). In the latter case, by the ligase activity of BAL, an important building block in the synthesis of cytoxazone was synthesized as a cross condensation product of *p*-anisaldehyde and dimethoxyacetaldehyde (Scheme 3b). In both cases, the reactions were carried out by adding excess aliphatic aldehydes in order to promote the cross-condensation reaction.

In the present study, we performed carboligation reactions with free and immobilized enzymes. The reactions were carried out until the desired conversions were achieved. These representative reactions were screened with a different amount of immobilized enzyme for obtaining the maximum amount of product formation (the reaction was monitored by HPLC, see Table 1). 8U enzyme immobilized on 900 mg magnetic resin is used. The reaction was repeated with free enzyme upon obtaining the same results. The reactions performed with free enzyme were started with 20 U enzyme purified by Ni affinity chromatography and freshly applied for the reactions. To obtain the same yield with the immobilized system, the addition of fresh enzyme was necessary (8 U) as reported earlier.[10,11] The amount of the enzyme in the immobilized system seems to be decreased but the exact difference of the enzyme amount cannot be defined in the present study.

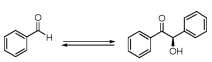
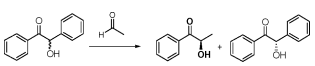
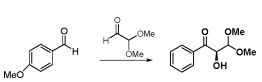
## Conclusions

In the present work, we efficiently immobilized BAL on magnetic particles and used them as a heterogeneous catalyst for some representative BAL-mediated carboligation reactions.




**Scheme 3** Immobilized BAL mediated reactions: (a) kinetic resolution of racemic benzoin with the lyase activity of the BAL resulting in cross condensation with acetaldehyde;[9] (b) synthesis of (*R*)-2-hydroxy-3,3-dimethoxy-1-(4-methoxyphenyl)propan-1-one, which is the cross condensation product of *p*-anisaldehyde with dimethoxy acetaldehyde.

**Table 1** Yields and enantiomeric excess (ee) values for the immobilized BAL catalyzed reactions

| Reaction type | Reaction mode[a] | Yield(%) | ee (%) | Recovery [(*S*)-benzoin] yield(%) | ee (%) | Time |
|---|---|---|---|---|---|---|
|  | Free | 89 | >99 | | | 40 min |
| | Immobilized | 85 | >99 | | | 40 min |
|  | Free | 47 | >99 | 44 | >99 | 48 h |
| | Immobilized | 44 | >99 | 43 | >99 | 48 h |
|  | Free | 85 | >99 | | | 24 h |
| | Immobilized | 83 | >99 | | | 24 h |

[a] From the same source of crude extract

These magnetically responsive particles can be used for the direct immobilization and easy separation of enzymes from a crude extract in order to obtain pure immobilized enzyme as well as a direct use for catalytical purposes. In addition, two pharmaceutically important representative reactions were performed with this system, in which the products were obtained in high chemical and optical yields. Additionally, the reusability of the immobilized enzyme (minimum 5 repeats without losing considerable activity) is another advantage compared to free enzyme. As a result of the present study, we hereby present a new biocatalyst system for BAL mediated acyloin reactions *via* C–C bond formation and bond breaking reactions with a high optical and chemical yield (ee > 99%). Our studies are now focusing on the increase of the surface/volume ratio of micron scale silica coated superparamagnetic resin in order to thereby increase the capacity of particles and for use as an application for new TPP dependent enzymes and enantioselective carboligation reactions.

## Experimental

### General remarks

Tetraethylorthosilicate (TEOS) was purchased from Aldrich and was used without any purification. *N*α*N*α-bis(carboxymethyl)-L-lysine hydrate (NTA) and 3-glycidoxypropyltrimethoxysilane (GPTMS) were purchased from FLUKA® and were used without any purification. Other reagents were commercially available analytical grade products.

All of the chemicals that were used in the immobilization studies were purchased from SIGMA-ALDRICH®. The *E. coli* BL21 (DE3) pLysS strain that was used to produce the recombinant BAL was purchased from Invitrogen®. For the carboligation reactions, the substrates benzaldehyde, acetaldehyde, racemic benzoin, and dimethoxy acetaldehyde were obtained from Sigma®. Reactions with free enzyme are carried out according to the literature procedure.[1,10]

### Analytics

The magnetic behavior of the iron oxide–silica nanocomposites was studied using a vibrating sample magnetometer (VSM) (Cryogenic Q-3398, England). The magnetic hysteresis curves were recorded at 300 K and in fields up to 7 T.

The transmission electron microscopy (TEM) images were performed using a JEOL 3010, Japan, microscope operating at 300 kV. The samples for TEM were prepared by mounting iron oxide–silica nanocomposite powder on SPI double copper grids 100/200.

The BAL-catalyzed reactions were monitored by thin layer chromatography (TLC) on silica gel (E. Merck, Darmstad). The detection of spots was performed by both UV-absorption and phosphomolybdicacid (PMA). The products synthesized were identified by $^{1}$H-NMR and the $^{13}$C-NMR spectra were recorded by BRUKER DPX 400 MHz by using tetramethylsilane (TMS) as an internal standard and deutero-chloroform as a solvent. The reactions were followed by HPLC analysis (Agilent 1100 series).

### Synthesis of γ-$Fe_2O_3$–silica nanocomposites[9]

An ethanolic solution (Ethanol, Carlo Erba, 99%) of iron nitrate nonahydrate ($Fe(NO_3)_3{\cdot}9H_2O$, Aldrich, 98%) was mixed with tetraethoxysilane (TEOS, Aldrich, 98%) to obtain the starting sol. The molar ratio of TEOS : Et-OH : water : $HNO_3$ was 0.23 : 1 : 0.5 : 0.001. The hydrolysis reaction was only promoted by the hydration water of the salt. The sol was stirred for 15 min. The surface/volume (S/V) ratio, which is defined as the ratio of the evaporation surface (the cross sectional area of the vessel) by the volume of the sol, was selected as 0.04 for this study. After adding 2.38 ml of the sol into each of the vessels, they were closed using a seal with a small punched hole and then heated in an oven set at the gel point of 70 °C. The calcination of the fresh gels was carried out at 400 °C under non-flowing air for 4 h, in which the calcinated gels were subsequently powdered.

### Surface modification of γ-$Fe_2O_3$–silica nanocomposite particles

First, the silane solutions of GPTMS–NTA were prepared. To prepare the GPTMS–NTA silane solution, 0.6 g NTA was dissolved in 100 ml of deionized water in a flask bottle with three openings, and the pH value of this solution was tuned to 11 using 10 M NaOH. The bottle containing the NTA solution was placed in the ice-bath at 0 °C, and subsequently 0.18 ml of the GPTMS solution was gradually added to the NTA solution while stirring. The mixed solution of GPTMS–NTA was heated to 65 °C and was kept at this temperature for 6 h while stirring continuously, in which the temperature of the GPTMS–NTA solution was again decreased to 0 °C. Additional 0.18 ml of GPTMS was added to the GPTMS–NTA solution while stirring, and then the temperature of the solution was increased to 65 °C and kept at this temperature for another 6 h. The pH value of the GPTMS–NTA solution was adjusted to 6 using HCl.

1.0 g of γ-$Fe_2O_3$–silica nanocomposite particles were added to the prepared GPTMS–NTA solutions, in which the temperature of the resulting suspension was raised to 95 °C and kept at this temperature while stirring. Subsequently, the surface modified γ-$Fe_2O_3$–silica nanocomposite particles were separated from the suspension by using a permanent magnet and washed with deionized water three times. The surface modified γ-$Fe_2O_3$–silica nanocomposite particles were labeled as γ-$Fe_2O_3$–silica–GPTMS–NTA particles.

### Attachment of metal ions ($Co^{2+}$) to the γ-$Fe_2O_3$–silica–GPTMS–NTA particles

0.1 g of γ-$Fe_2O_3$–silica–GPTMS–NTA particles were mixed with 10 ml of 1 M aqueous solution of $CoCl_2$, in which the resulting mixture was shaken for 1 h to reach equilibrium. After charging the γ-$Fe_2O_3$–silica–GPTMS–NTA particles with $Co^{2+}$, they were separated using a permanent magnet, and washed with 5 ml of deionized water six times. The excess unbound $Co^{2+}$ metal ions were removed with water. The $Co^{2+}$ charged particles were labeled as γ-$Fe_2O_3$–silica–GPTMS–NTA–$Co^{2+}$.

### Preparation of a cell free extract and the isolation of BAL from a recombinant *E. coli* strain overexpressing BAL

*E. coli* BL21(DE3) pLysS containing the pUC19-$BAL_{HIS}$ construct was cultured at 37 °C in 1.65 L Luria broth (LB) in a 2 L fermentor (New Brunswick BioFlo110). 6 h after the induction with isopropyl-β-D-thiogalactopyranoside (IPTG), cells were harvested by centrifugation. Pelleted cells were transferred to a Petri dish

and lyophilized for 36 h. For the free enzyme preparation, crude extract was applied to a $Ni^{2+}$–NTA affinity column (Invitrogen®) and desalting column (Amersham) and the eluent containing pure BAL was used in the carboligation reactions.

### Activity assays

One unit of ligase activity of BAL is defined as the amount of enzyme that catalyzes the formation of 1 μmol of benzoin per minute under the standard conditions (30 °C, pH: 7.8). With free and immobilized system initial rates of BAL, the catalyzed benzoin formation reactions were determined by HPLC analysis (Nucleodur C18, 1 mL $min^{-1}$, 254 nm, retention time 12 min).

### Immobilization of BAL to superparamagnetic particles

Superparamagnetic γ-$Fe_2O_3$ (maghemite)–silica nanocomposite particles were synthesized by using the sol–gel method, in which the surface modification method was also applied for the immobilization of 6XHistidine tagged recombinant benzaldehyde lyase (BAL, EC 4.1.2.38).[8] 185 mg γ-$Fe_2O_3$ nanoparticles were washed with a lysis buffer (10 mM imidazole, 100 mM NaCl in 50 mM potassium phosphate buffer) and incubated for a couple of hours at 4 °C in order to enable the silica coat of nanoparticles to swell. Equilibrated resin was settled for 1–2 min by the aid of a magnet, in which the supernatant was removed by pipetting. 200 mg lyophilized crude extract was dissolved in a 30 mL lysis buffer. After sonication, the slurry was centrifugated and the supernatant was filtered through a 0.45 μm filter. 2 mL of filtered crude was diluted to 5 mL and incubated with magnetic resin by gentle mixing (90 rpm) at 4 °C for 20 min to immobilize the histidine tagged BAL. The amount of crude loaded was estimated according to the saturation concentration for the resin. After the protein was immobilized, the resin was settled as described before and washed with a lysis buffer twice.

The amount of immobilized BAL was determined by measuring the protein content in the eluted enzyme solution (200 mM imidazole, 100 mM NaCl, eluted protein in 50 mM potassium phosphate buffer (pH: 7.8) and by the colorimetric method at 595 nm by using the SIGMA Bradford reagent with bovine serum albumin as a standard.[11] SDS page analysis was performed by the method of Leammly (1970).[14]

### Representative reaction with superparamagnetic particles: synthesis of 2-hydroxy-1,2-diphenylethan-1-one (*R*)-2

BAL immobilized resin was equilibrated with a reaction buffer containing 0.25 mM TPP, 2.5 mM $MgSO_4$, 25% DMSO in 50 mM potassium phosphate buffer at pH: 7.8. The mixture was incubated *via* gentle shaking (90 rpm), at 25 °C for 1 min, and then the reaction was started by adding benzaldehyde (50 mg, 0.5 mmol). The reaction was monitored by HPLC (Nucleodur C18, 1 mL $min^{-1}$, 254 nm, retention time 12 min., 45% acetonitrile, 0.5% acetic acid, and 54.5% water). After 40 min. (checked by TLC), the reaction mixture was extracted with chloroform (3 × 50 mL).

After drying the collected organic phase over $MgSO_4$, removal of the solvent under reduced pressure gave the crude product, which was then purified by flash column chromatography (EtOAc–hexane 1 : 3) to give 42.5 mg (85%, 99% ee) of (*R*)-**2**: mp 135 °C Lit.[1], 133–134 °C for (*R*) enantiomer; $[\alpha]_D^{22} = -112.1$ (*c* 1.5, $CH_3COCH_3$) [lit.[1] $[\alpha]_D^{22} = -113.8$ (*c* 1.5, $CH_3COCH_3$). HPLC: (Chiralpak AD) $R_t(R) = 27.1$ min; $R_t(S) = 34.5$ min; $^1$H NMR (400 MHz, $CDCl_3/CCl_4$): $\delta$ 7.82 (d, $J$ = 7,8 Hz, 2H), 7.44 (t, $J$ = 7.5, 1H), 7.30 (t, $J$= 7.6), 7.16–7.23 (m, 5H), 5.73 (d, $J$ = 5.9 Hz, 1H), 4.42 (d, $J$ = 5.9 Hz, 1H); $^{13}$C NMR (100 MHz, $CDCl_3/CCl_4$): 198.7, 139.1, 133.8, 133.6, 129.1, 129.0, 128.6, 128.5, 127.7, 76.2.

### Synthesis of 2-hydroxy-1,2-diphenylethan-1-one (*S*)-2 and (*R*)-2-hydroxy-1-phenylpropanone [(*R*)-3] from racemic benzoin and acetaldehyde[1]

According to the procedure for the synthesis of (*R*)-**2**, *rac.* benzoin (50 mg, 0.23 mmol) and 74 mg (0.7 mmol) acetaldehyde were dissolved in the 20 mL reaction medium that was prepared for the 900 mg BAL immobilized resin. The reaction was performed at 30 °C and acetaldehyde (0.2 mmol) was added (30 and 120 min). The reaction was followed by TLC and stopped at 48 h by adding chloroform (50 mL). The mixture was extracted twice with chloroform (2 × 50 mL). After drying the collected organic phase over $MgSO_4$, removal of the solvent under reduced pressure gave the crude product mixture, which was then purified by flash column chromatography to give the desired compounds (*S*)-**2** and (*R*)-**3** (EtOAc–hexane 1 : 3).The determination of the enantiomeric excess was performed by HPLC analysis (Chiralpak AD, 90 : 10 hexane–isopropanol, 1 mL $min^{-1}$, 254 nm, retention time for (*R*)-2-hydroxy-1-phenylpropanone: 12.45, for (*S*)-benzoin: 33.35). (*S*)-**2**: yield 10.5 mg (43%), 99% ee; $[\alpha]_D^{22} = 109.4$ (*c* 1.5, $CH_3COCH_3$). (*R*)-**3**: Viscous oil: $[\alpha]_D^{22} = 83.5$ (*c* 2.0, $CHCl_3$), Lit.[1] $[\alpha]_D^{22}$ : 85.1 (*c* 2.0, $CHCl_3$). $^1$H NMR (400 MHz, $CDCl_3/CCl_4$): $\delta$ 7.90 (dd, $J$ = 1.4, 8.2 Hz, 2H), 7.40–7.60 (m, 3H), 5.13 (q, $J$ = 6.0 Hz, 1H), 3.80 (br.s, 1H), 1.41 (d, $J$ = 6.0 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3/CCl_4$): $d$ = 202.7, 134.4, 134.0, 128.9, 128.7, 69.2, 22.0.

### Synthesis of (*R*)-2-hydroxy-3,3-dimethoxy-1-(4-methoxyphenyl)propan-1-one (*R*)-5[9]

According to the procedure for the synthesis of (*R*)-**2**, *p*-anisaldehyde (50 mg, 0.36 mmol) and dimethoxy acetaldehyde (112 mg, 1 mmol) were dissolved in the 30 mL reaction medium that was prepared for the 900 mg BAL immobilized resin added and incubated at 30 °C *via* gentle shaking (90 rpm). After 2 h, 18.7 mg (0.18 mmol) dimethoxy acetaldehyde was added to the medium. The reaction was followed by TLC and was stopped at 24 h. The reaction mixture was extracted with chloroform (3 × 50 mL). After drying the collected organic phase over $MgSO_4$, removal of the solvent under reduced pressure gave the crude product, which was then purified by flash column chromatography (EtOAc–hexane 1 : 3) to give 72 mg (83%, 99% ee) (*R*)-**5** as a viscous oil. $[\alpha]_D^{22} = -13.7$ (*c* 0.5, $CHCl_3$); HPLC (Chiralcel OD, UV detection at 254 nm, 97 : 3 hexane–2-propanol, flow rate 0.8 mL $min^{-1}$): $R_t(R)$= 40.7 min; $R_t(S)$= 45.3 min; $^1$HNMR: 3.30 (s, 3H), 3.39 (s, 3H), 3.62 (d, $J$ = 7.2, 1H), 3.82 (s, 3H), 4.33 (d, $J$ = 3.5, 1H), 5.0 (dd, $J$ = 3.5, 7.2, 1H), 6.92 (d, $J$ = 8.7, 2H), 7.97 (d, $J$ = 8.7, 2H); $^{13}$C NMR: 55.4, 55.7, 56.7, 73.9, 106.4, 113.9, 128.3, 132.1, 164.4, 197.0.

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