# **Electronic Supplementary Information**

### for

# **Co-immobilization of Metal and Enzyme into Hydrophobic Nanopores for Highly Improved Chemoenzymatic**

# **Asymmetric Synthesis**

Liya Gao<sup>a</sup>, Zihan Wang<sup>a</sup>, Yunting Liu<sup>a</sup>, \*, Pengbo Liu<sup>a</sup>, Shiqi Gao<sup>a</sup>, Jing Gao<sup>a</sup>, and Yanjun Jiang<sup>a, b \*</sup>

<sup>a</sup>School of Chemical Engineering and Technology, Hebei University of Technology, 8 Guangrong Road, Hongqiao District, Tianjin, 300130, PR China <sup>b</sup>National-Local Joint Engineering Laboratory for Energy Conservation of Chemical Process Integration and Resources Utilization, Hebei University of Technology, Tianjin, 300130, China

\* Corresponding authors

Yunting Liu, Email: ytliu@hebut.edu.cn, Tel: +86-22-60204945.

Yanjun Jiang, Email: yanjunjiang@hebut.edu.cn, Tel: +86-22-60204945.

#### **Table of Contents**

Cemicals and materials	S3
Analytical methods	S3
Experimental section	S3
Synthesis of DSNs	S3
Synthesis of DONs	S3
Synthesis of DON@Pd	S4
Synthesis of DON@Pd-CALB@PDA	S4
Expression and purification of ADH	S4
The fabrication of DON@Pd-ADH@PDA	S5
Dynamic Kinetic Resolution of Chiral Amines	S5
Chemoenzymatic synthesis of chiral benzyl alcohols	S7
Results and Discussion	S10
Characterization of catalysts	S10
Supporting Tables	S13
Table S1. Optimization of the Racemization Conditions	S13
Table S2. Effect of different additives and catalyst on the reaction	S13
Table S3. Optimization of the aqueous chemoenzymatic synthesis of chiral alcohols	S14
NMR Spectra of Compounds	S15
GC chromatogram of Compounds	S24
References	S35

#### **Chemicals and materials**

All chemicals were purchased from J & K, Acros and Aldrich, and were used as received. Anhydrous THF, toluene was distilled from sodium benzophenone ketyl. Hydrogen gas (99.999%) was purchased from Boc Gas Inc., Tianjin. CALB was purchased from Novozymes (China) Biotechnology Co., Ltd. Nicotinamide adenine dinucleotide (NADH) were purchased from Aladdin (Shanghai, China).

#### **Analytical methods**

SEM images of DSNs and DONs were recorded on Nova Nano SEM450 field-emission microscope. TEM images of DON@Pd and DON@Pd@PDA were recorded on Talos F200S. Inductively coupled plasma-optical emission spectroscopy (ICP-OES) was carried out on Optima 8300. XPS spectra were collected by a Thermo Scientific K-Alpha X-ray photoelectron spectrometer. Fourier-transform infrared spectroscopy (FT-IR) characterization was obtained on Bruker VECTOR22 spectrometer. Shimadzu's GC2010 gas chromatograph is used for gas phase detection TLC was carried out using Kieselgel 60 F254 (Merck) sheets. NMR spectra were recorded on a Bruker AV 400 spectrometer at 400 MHz (<sup>1</sup>H NMR and <sup>13</sup>C NMR). Chemical shifts were reported in ppm relative to internal TMS for <sup>1</sup>H NMR data, respectively. Data are presented in the following space: chemical shift, multiplicity, coupling constant in hertz (Hz), and signal area integration in natural numbers.

#### **Experimental section**

#### **Synthesis of DSNs**

Dendritic silica nanoparticles (DSNs) were prepared by the continuous phase microemulsion method. The detailed procedure for synthesis of DSNs was shown as follows: Firstly, 1 g cetyltrimethyammniumbromide (CTAB) and 1 g *n*-butanol were dissolved in 30 g urea solution (0.4 M); Secondly, 12 g cyclohexane was added and stirred to form a microemulsion solution; next, 2 g tetraethyl orthosilicate (TEOS) was added to the mixture and stirred at 25 °C for 30 min; Lastly, the mixture was stirred at 70 °C for 20 h. The products were collected and calcined at 550 °C for 5 h to remove the template.

#### Synthesis of DONs

Dendritic organosilica nanoparticles (DONs) were prepared based on the continuous phase microemulsion method. Firstly, 1.25 g cetyltrimethyammniumbromide (CTAB), 1.25 g *n*-butanol

and 5 g cyclohexane were dissolved in 100 g urea solution (0.4 M) and then the mixture was ultrasonicated for 30 min; Secondly, the solution of tetraethyl orthosilicate (TEOS, 0.875 g) and bis(triethoxysilyl)ethane (BTSE, 0.375 g) was added dropwise to the above mixture and stirred at 25 °C for 30 min; Next, the mixture was stirred at 70 °C for 24 h. The products were washed with ethanol and water for several times. Finally, DONs were redispersed in 250 mL of acetone and refluxed at 80 °C for 48 h to remove the templates and then washed with ethanol and dried at room temperature.

#### Synthesis of DON@Pd

Immobilization of Pd nanoparticles (Pd NPs) in the channels was achieved via in situ growth approach. Typically, 56 mg of DON was ultrasonically dispersed in 10 mL of ultrapure water and the mixture was stirred for 15 min at 30 °C. Next, a solution of sodium tetrachloropalladate (27.29 mg) for DON@Pd (15%); (17.20 mg) for DON@Pd (10%) and (8.14 mg) for DON@Pd (5%) was added dropwise to the reaction mixture and further stirred for 4 h at 30 °C. Then, NaBH<sub>4</sub> (10 equiv.) were added to the above solutions with stirring for 2 h. The resulting solids were isolated by centrifugation, and washed with water and ethanol, dried under vacuum at 60 °C for 12 h, obtaining DON@Pd.

#### Synthesis of DON@Pd-CALB@PDA

Typically, DON@Pd (20 mg) was dispersed in 2.5 mL PBS buffer (100 mM, pH 7), then added 2.5 mL CALB. The mixture was stirred for 6 h. The mixture was washed by PBS buffer (100 mM, pH 7) for 3 times. Then, the catalyst was dried by a vacuum freeze dryer for 12 h

The obtained DON@Pd-CALB (10 mg) was dispersed in 10 mL Tris-HCl buffer solution (50.0 mM, pH 8.5). After stirring for 5 min at room temperature, a certain amount of dopamine (2.0 mg/mL) was added to the above solution. The coating process was maintained for 4, 8 and 12 h. Correspondingly, the PDA coating layer was gradually formed on the surface of the DON@Pd-CALB. Subsequently, the solid was separated by centrifugation and washed with Tris-HCl buffer solution for several times. Finally, the DON@Pd-CALB@PDA was obtained after drying under vacuum at 60 °C for 12 h.

#### The expression and purification of ADH

The gene for the ADH from *Rhodococcus ruber* was transferred in *E. coli* by pET-28a. The *E. coli* was precultured in 10 ml of Luria-Bertani (LB) medium with ampicillin antibiotics at 37 °C for 16

h. Then, the cells were transferred into 50 mL of LB medium with ampicillin antibiotics and incubated at 37 °C until  $OD_{600}$  (optical density of the cell suspension measured at 600 nm) reached 0.6-0.8. Subsequently, 1mmol/L IPTG was added to induce protein expression. The recombinant cell pellets were obtained by centrifugation. The cell pellets were crushed by homogenizer and purified by protein purifier.

#### The fabrication of DON@Pd-ADH@PDA

Firstly, 10 mg DON@Pd was uniformly dispersed in 3 mL Tris-HCl buffer (350 mM, pH 8) by ultrasonic, 1 mg purified ADH was added and stirred slowly at 30°C for 8 hours. Then, the mixture was centrifuged and washed by buffer three times, and the DON@Pd-ADH was dried by a vacuum freeze dryer. Next, the DON@Pd-ADH was coated with PDA by the above-mentioned method. The catalyst was dried by a vacuum freeze dryer for 12 h.

#### **Dynamic Kinetic Resolution of Chiral Amines**



The mixture of amines (0.3 mmol), DON@Pd-CALB (15 mg), dry Na<sub>2</sub>CO<sub>3</sub> (0.3 mmol), and ethyl methoxyacetate (0.6 mmol) in dry toluene (2 mL) was added to a dry Schlenk tube. Pentadecane was added as internal standard. The Schlenk tube was evacuated three times and filled with hydrogen gas (0.1 atm) and the reaction was stirred at 60 °C. We monitored the progress of the reaction by thin layer chromatography. After the reaction was completed, the mixture was diluted with ethyl acetate and washed with saturated sodium bicarbonate and brine. The organic phase was dried using Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated in vacuo. The crude products were purified by column chromatography (SiO<sub>2</sub>, petroleum ether/EtOAc 100:0 to 0:100) and the yields and the values of ee were determined by GC.

(*R*)-2-methoxy-N-(1-phenylethyl)acetamide (2a)



mL/min); injection temp, 180 °C; initial column temperature 125 °C, then progress rate, 3 °C/min; final column temperature, 160 °C for 15 min;  $t_R = 9.489$  min (minor) and 9.713 min (major).

#### (*R*)-2-methoxy-N-(1-(p-tolyl)ethyl)acetamide (**2b**)





gas, N<sub>2</sub> (flow 30 mL/min); injection temp, 180 °C; initial column temperature 125 °C, then progress rate, 3 °C/min; final column temperature, 160 °C for 15 min;  $t_R = 11.873$  min (minor) and 12.380 min (major).

#### (*R*)-2-methoxy-N-(1-(3-methoxyphenyl)ethyl)acetamide (2c)



Colorless oil, 93% yield, 96% ee, <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.27 (t, J = 7.8 Hz, 2H), 7.11-6.44 (m, 4H), 5.14 (dd, J = 13.9, 6.8 Hz, 1H), 3.91 (t, J = 10.0 Hz, 2H), 3.86-3.76 (m, 3H), 3.41 (s, 3H), 1.51 (d, J = 6.9 Hz, 3H). GC conditions: Agilgent CP-Chirasil Dex CB (df = 0.25

 $\mu$ m, 0.32 mm i.d. × 25 m); carrier gas, N<sub>2</sub> (flow 30 mL/min); injection temp, 180 °C; initial column temperature 125 °C, then progress rate, 3 °C/min; final column temperature, 160 °C for 15 min; t<sub>R</sub> = 17.077 min (minor) and 17.879 min (major).

(R)-2-methoxy-N-(4-phenylbutan-2-yl)acetamide (2d)



conditions: Agilgent CP-Chirasil Dex CB (df = 0.25  $\mu$ m, 0.32 mm i.d. × 25 m); carrier gas, N<sub>2</sub> (flow 30 mL/min); injection temp, 180 °C; initial column temperature 125 °C, then progress rate, 3 °C/min; final column temperature, 160 °C for 15 min; t<sub>R</sub> = 16.711 min (minor) and 17.340 min (major).

(*R*)-2-methoxy-N-(1,2,3,4-tetrahydronaphthalen-1-yl)acetamide (2e)



i.d. × 25 m); carrier gas, N<sub>2</sub> (flow 30 mL/min); injection temp, 180 °C; initial column temperature 125 °C, then progress rate, 20 °C/min to 150 °C, then progress rate, 0.2 °C/min; final column temperature, 163 °C for 5 min;  $t_R = 21.501$  min (minor) and 22.059 min (major).

#### (R)-N-(2,3-dihydro-1H-inden-1-yl)-2-methoxyacetamide (2f)



White solid, mp 50–52 °C 92% yield, 99% ee, <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.30-7.12 (m, 5H), 6.75 (s, 1H), 5.54 (d, *J* = 8.0 Hz, 1H), 3.96 (s, 2H), 3.44 (d, *J* = 32.3 Hz, 3H), 2.95-2.83 (m, 1H), 2.82-2.74 (m, 1H), 1.94-1.64 (m, 2H). GC conditions: Agilgent CP-Chirasil Dex CB (df = 0.25 μm, 0.32 mm

i.d. × 25 m); carrier gas, N<sub>2</sub> (flow 30 mL/min); injection temp, 180 °C; initial column temperature 125 °C, then progress rate, 20 °C/min to 150 °C, then progress rate, 0.2 °C/min; final column temperature, 163 °C for 5 min;  $t_R = 15.583$  min (minor) and 15.735 min (major).

#### Chemoenzymatic synthesis of chiral benzyl alcohols



S-(*tert*-butyl) ethanethioate (4, 0.5 mmol), arylboronic acids (5, 1.7 equiv.), copper-(I)-thiophene-2-carboxylate (CuTC, 1.5 equiv.) and catalyst (50 mg) were added to a 25 ml reaction flask containing a 10 mL mixture solution of Tris-HCl buffer (pH 8.0, 50 % v/v), NADP<sup>+</sup>, *n*-heptane (20 % v/v) and *i*-PrOH (30 % v/v). The mixture was thermostated at 37 °C for 24 h. The products were purified by column chromatography using petroleum ether/ethyl acetate (5:1) as eluent. The values of ee were determined by GC.

After the reaction was completed, the mixture was centrifuged to separate the catalyst and then washed with buffer to remove the remaining substrate and product. The catalyst was then used in a

new cycle. The experimental results were shown in Figure S7. The yields and the values of ee were determined by GC.

#### (S)-1-phenylethanol (6a)

Colorless oil, bp 224 °C, 86% yield, 99% ee. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.51-7.25 (m, 5H), 5.18-4.56 (m, 1H), 1.88 (d, J = 89.8 Hz, 1H), 1.63-1.32 (m, 3H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  145.84, 128.53, 127.51, 125.42, 70.44, 25.19. GC conditions: CP-Chirasil Dex CB (df = 0.25 µm, 0.32 mm i.d. × 25 m); carrier gas,

N<sub>2</sub> (flow 30 mL/min); injection temp, 250 °C; initial column temperature 80 °C, then progress rate, 5 °C/min to 160 °C, then progress rate, 10 °C/min; final column temperature, 220 °C for 8 min;  $t_R$  = 10.353 min (minor) and 10.565 min (major).

#### (S)-1-(4-fluorophenyl)ethanol (6b)



25.30 (s). GC conditions: CP-Chirasil Dex CB (df = 0.25  $\mu$ m, 0.32 mm i.d. × 25 m); carrier gas, N<sub>2</sub> (flow 30 mL/min); injection temp, 250 °C; initial column temperature 80 °C, then progress rate, 5 °C/min to 160 °C, then progress rate, 10 °C/min; final column temperature, 220 °C for 8 min; t<sub>R</sub> = 11.067 min (minor) and 11.484 min (major).

#### (S)-1-(4-chlorophenyl)ethanol (6c)



0.32 mm i.d. × 25 m); carrier gas, N<sub>2</sub> (flow 30 mL/min); injection temp, 250 °C; initial column temperature 80 °C, then progress rate, 5 °C/min to 160 °C, then progress rate, 10 °C/min; final column temperature, 220 °C for 8 min;  $t_R = 15.124$  min (minor) and 15.735 min (major).

#### (S)-1-(4-bromophenyl)ethanol (6d)

Colorless oil, bp 253.3 °C, 81% yield, 98% ee. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  

$$\delta$$
 7.47 (d,  $J = 8.2$  Hz, 2H), 7.24 (s, 2H), 4.87 (q,  $J = 6.3$  Hz, 1H), 1.82 (s,  
1H), 1.47 (d,  $J = 6.4$  Hz, 3H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  143.98,  
130.78, 126.38, 120.38, 69.01, 24.48. GC conditions: CP-Chirasil Dex CB (df = 0.25 µm, 0.32 mm  
i.d. × 25 m); carrier gas, N<sub>2</sub> (flow 30 mL/min); injection temp, 250 °C; initial column temperature  
80 °C, then progress rate, 1 °C/min; final column temperature, 220 °C for 5 min; t<sub>R</sub> = 48.440 min  
(minor) and 49.877 min (major).

#### (S)-1-[4-(trifluoromethyl)phenyl]ethanol (6e)



122.82, 69.85, 25.41. GC conditions: CP-Chirasil Dex CB (df = 0.25  $\mu$ m, 0.32 mm i.d. × 25 m); carrier gas, N<sub>2</sub> (flow 30 mL/min); injection temp, 250 °C; initial column temperature 80 °C, then progress rate, 1 °C/min; final column temperature, 220 °C for 5 min; t<sub>R</sub> = 26.968 min (minor) and 29.940min (major).

(S)-1-(4-methylphenyl)ethanol (6f)



conditions: CP-Chirasil Dex CB (df = 0.25  $\mu$ m, 0.32 mm i.d. × 25 m); carrier gas, N<sub>2</sub> (flow 30 mL/min); injection temp, 250 °C; initial column temperature 80 °C, then progress rate, 5 °C/min to 160 °C, then progress rate, 10 °C/min; final column temperature, 220 °C for 8 min; t<sub>R</sub> = 16.28 min (minor) and 16.76 min (major).

### **Characterization of catalysts**



**Figure S1** SEM images of DONs synthesized at various BTSE and TEOS molar ratio: (a) 0/1, (b) 1/9, (c) 3/7.



**Figure S2** TEM images and particle size distribution of DON@Pd with various Pd loading amount: (a-c) 4.8%, (d-f) 9.6%, (g-i) 15.1%.



Figure S3 SEM image of DON@Pd-CALB@PDA synthesized at 35 mM dopamine.



concentration.

Figure S4 Wide-angle XRD patterns of the DON@Pd-CALB and DON@Pd-CALB@PDA.



**Figure S5** (a) XPS spectrum of the DON@Pd-CALB@PDA. (b) The Pd 3d core level peak of the DON@Pd-CALB@PDA.



Figure S6 Reusability experiment results of the DON@Pd-ADH@PDA

#### Supporting Tables

Table S1. Optimization of the Racemization Conditions
---

	N 	H <sub>2</sub>	H <sub>2</sub> (0.1 a la <sub>2</sub> CO <sub>3</sub> (1. Catalyst (2	atm) 0 equiv) mol%)	I	NH <sub>2</sub>	
			Toluer	le			
	( <i>R</i> )- <b>1a</b>				( <i>Rac</i> )- <b>1a</b>		
Entry	Catalysts	Nanoparticle size (nm)	Time (h)	Temperature (°C)	Selectivity	ee (%) <sup>b</sup>	Ref.
1	Pd/C	-	24	70	0	-	1
2	Pd/BaSO <sub>4</sub>	-	24	70	81	2	1
3	Pd/AlO(OH)	3-5	24	70	85	2	2
4	Pd/PP-SiO <sub>2</sub>	11	24	70	89	6	3
5	DSN@Pd	1.8	8	70	>95	3	
6	DON@Pd	1.8	4	70	>96	2	
7	DON@Pd	4.0	12	70	>95	2	
8	DON@Pd	11	24	70	>95	7	
9	DON@Pd	1.8	12	60	>95	1	
10	DON@Pd	1.8	24	50	>95	4	

<sup>*a*</sup> Reaction conditions: (*R*)-1a (0.3 mmol), Catalyst (2 mol%), Na<sub>2</sub>CO<sub>3</sub> (30 mg), toluene (2 mL), reacting 8 h. <sup>*b*</sup> Determined by GC and pentadecane as internal standard.

Table S2. Effect of different additives and catalyst on the reaction
--

	$ \begin{array}{c}                                     $	H <sub>2</sub> (0.1 atm) Na <sub>2</sub> CO <sub>3</sub> (1 equiv) Catalyst (2 mol%) Toluene	$\rightarrow$	HN Za	
Entry	Catalyst	temperature (°C)	Time (h)	Yield (%) <sup><math>b</math></sup>	ee (%) <sup>b</sup>
1	DON@Pd-CALB	60	12	98	99
2	DON@Pd/DON@CALB	60	12	66	91
3	DSN@Pd-CALB	60	12	72	98
4	DSN@Pd-CALB	60	24	94	98
5	DSN@Pd-CALB	70	12	97	98
6	DON@Pd-CALB	70	6	98	98
7	DON@Pd-CALB	50	24	80	98
8	DON@Pd-CALB@PDA	60	24	83	98

<sup>&</sup>lt;sup>*a*</sup> Reaction conditions: All reactions were carried out in dry toluene (2.0 mL) under 0.1 atm of hydrogen gas using 1-phenylethylamine (0.3 mmol), ethyl methoxyacetate (0.6 mmol), catalyst (0.1 equiv), additive (1 equiv). <sup>*b*</sup> Determined by GC and pentadecane as internal standard.

Entry	Conditions	Yield $(\%)^b$	ee (%) <sup>c</sup>
1	Tris-HCl buffer, NADP <sup>+</sup> , <i>i</i> -PrOH (30% v/v)	68	99
2	Tris-HCl buffer, NADP <sup>+</sup> , <i>n</i> -heptane (20% v/v), <i>i</i> -PrOH (30% v/v)	86	99
3	Tris-HCl buffer, NADP <sup>+</sup> , glucose (15 mM), glucose dehydrogenase (1 U mL <sup>-1</sup> )	65	99
4	Tris-HCl buffer, <i>n</i> -heptane (20% v/v), NADP <sup>+</sup> , glucose (15 mM), glucose dehydrogenase (1 U mL <sup>-1</sup> )	72	99
5 <sup>d</sup>	Tris-HCl buffer, NADP <sup>+</sup> , <i>n</i> -heptane (20% v/v), <i>i</i> -PrOH (30% v/v)	26	96
6 <sup>e</sup>	Tris-HCl buffer, NADP <sup>+</sup> , <i>n</i> -heptane (20% v/v), <i>i</i> -PrOH (30% v/v)	46	99

Table S3. Optimization of the aqueous chemoenzymatic synthesis of chiral alcohols<sup>a</sup>

<sup>*a*</sup> All reactions were carried out for 24 h using S-(tert-butyl) ethanethioate (0.5 mmol, 50 mM), phenylboronic (1.7 equiv.), CuTC (1.5 equiv.) and DON@ADH@PDA@Pd (50 mg); <sup>*b*</sup> Isolated yield; <sup>*c*</sup> The values of ee were determined by GC; <sup>*d*</sup> using DON@Pd@PDA@ADH as catalyst; <sup>*e*</sup> using DSN@ADH@PDA@Pd as catalyst.

### NMR Spectra of Compounds





**S16** 



























# GC chromatogram of chiral benzyl alcohols



# 2-methoxy-N-(1-phenylethyl)acetamide

#	Time/min	Area	Height	Area%
1	9.489	4441	1334	0.065
2	9.713	6852891	1335746	99.935



### 2-methoxy-N-(1-(p-tolyl)ethyl)acetamide

2

12.380

679747

99.499

55143



### 2-methoxy-N-(1-(3-methoxyphenyl)ethyl)acetamide

# 2-methoxy-N-(4-phenylbutan-2-yl)acetamide



#	Time/min	Area	Height	Area%
1	16.711	10073	863	0.624
2	17.340	1604945	123637	99.376



### 2-methoxy-N-(1,2,3,4-tetrahydronaphthalen-1-yl)acetamide



### N-(2,3-dihydro-1H-inden-1-yl)-2-methoxyacetamide

# 1-phenylethanol



### 1-(4-fluorophenyl)ethanol



# 1-(4-chlorophenyl)ethanol



# 1-(4-bromophenyl)ethanol



# 1-[4-(trifluoromethyl)phenyl]ethanol



### 1-(4-methylphenyl)ethanol



#### References

- A.N. Parvulescu, P.A. Jacobs, D.E. De Vos, *Chem. Eur. J.*, 2007, **13**, 2034-2043.
   M.J. Kim, W.H. Kim, Y. K. Choi, J. Park, *Org. Lett.*, 2007, **9**, 1157-1159.
   A. N. Parvulescu, P. A. Jacobs, D. E. De Vos, *Appl. Catal.*, A 2009, **368**, 9-16.