Supplementary Information

Suzuki-Miyaura cross-coupling catalyzed by protein-stabilized palladium nanoparticles under aerobic conditions in water: application to a one-pot chemoenzymatic enantioselective synthesis of chiral biaryl alcohols

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Experimental Section

General information

Melting points were determined with a Büchi B-545 apparatus and are uncorrected. All of the reagents and solvents are commercially available and were used as purchased, without further purification. Compounds were purified on axially compressed columns, packed with SiO_2 25-40 μm (Macherey Nagel), connected to a Gilson solvent delivery system and to a Gilson refractive index detector, and eluting with *n*-hexane/AcOEt mixtures. ^{1}H NMR (400.13 MHz) and ^{13}C NMR (100.6 MHz) spectra were recorded with a Brüker Avance 400 spectrometer. Infrared (IR) spectra were recorded on a JASCO FT/IR-430 spectrophotometer. Mass spectra were determined with a QP2010 Gas Chromatograph Mass spectrometer (EI ion source). UV-Vis absorption spectra were recorded between 220-700 nm at rt with a spectrophotometer Varian Cary 50 and using a quartz cuvette with 0.1 cm path length. Transmission electron microscopy (TEM) analyses were made by Philips 208 transmission electron microscope (FEI Company) at 70 kV. The samples (10 μl) were applied to carbon coated copper grids (200-mesh) and after 30 s on the grid the samples were dried and visualized. In addition, some samples were negatively stained with (10 μl) phosphotungstic acid (PTA) 2% w/v solution buffered at pH = 7.3 to visualize the protein shell (Figure S1).

The enantiomeric excess was determined by enantioselective HPLC on the Chiralpak AS-H chiral stationary phase using the mixture *n*-hexane-2-propanol 95/5 (v/v) as a mobile phase.

Cloning, protein expression and purification

Recombinant *Thermosynechococcus elongatus* Dps (TeDps) protein was obtained and purified as described by Franceschini et al. (2006), removing the two ammonium sulfate cuts. Typically yield was 200 mg of Dps protein per 1 L batch. Protein concentration were determined spectrophotometrically on the basis of an ε molar of 19940 M^{-1} cm^{-1} (*M*w 214 kDa) at 280 nm.

Preparation of of palladium nanoparticles stabilized by a thermophilic DNA binding protein from starved cells (Pd_{np}/Te-Dps)

A Dps solution (0.23 μmol, 0.15 M in NaCl) was brought to pH 8.5 using 30 mM NaOH (TITRINO, Metrohm AG). K_2PdCl_4 (969 μg, 29.7 μmol) was added to the protein solution under stirring at room temperature for 30 min and then $NaBH_4$ (156 μg, 41.4 μmol) for over 15 min. Any possible aggregates of Dps and/or palladium particles produced during nanoparticles formation were removed by filtration through 0.22 μm syringe filters. The solution was dialyzed against 0.15 M NaCl and concentrated for the following analyses. The samples containing Pd_{np}/Te-Dps was checked using Supherose 6 gel-filtration column (GE Healthcare), equilibrated with 0.15 M NaCl.

Typical procedure for the Suzuki-Miyaura cross-coupling reaction using 0.05 mol% of Pd_{np}/Te-Dps

4-Iodobenzoic acid (62 mg, 0.25 mmol), 0.05 mol% of Pd_{np}/Te-Dps, o-tolylboronic acid (34 mg, 0.25 mmol.) in Tris (2 mL, pH = 8.9, 100 mM) were orbitally stirred for 24 h at 100 °C with a Heidolph Synthesis System. Then, after cooling, the liquid phase was extracted with CH_2Cl_2, the organic layer was washed with deionized water and dried over Na_2SO_4. The solvent was removed under vacuum and the residue was purified by chromatography (SiO_2, 35 g, *n*-Hexane/AcOEt/AcOH 96/3/1 v/v) to give 47.7 mg (90% yield) of **3a** m.p: 187.5-188.8 °C; IR (KBr) 2949, 2905, 1673, 1608, 1427, 1321, 1291 cm^{-1}; ^{1}H NMR ($DMSO_{d6}$) δ 8.02−7.99 (d, *J = 8.6 Hz*, 2H), 7.44-7.43(d, *J = 8.0 Hz*, 2H), 7.33-7.21 (m, 4H), 2.24 (s, 3H); ^{13}CNMR ($DMSO_{d6}$) δ 167.7, 146.24, 140.84, 135.17, 130.99, 129.86, 129.75, 129.73, 128.35, 126.56, 20.58. Anal Calcd for $C_{14}H_{12}O_{22}$ C, 79.22; H, 5.70; found C, 79.20; H, 5.68.

3b: m.p: 73.5-73.6 °C; IR (KBr) 2923, 2853, 1607, 1586, 1500, 1287, 1245, 806 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.68 (s, 4H), 7.58-7.56 (d, *J = 8.0 Hz*, 2H), 7.04-7.01 (d, *J = 8.0 Hz*, 2H); ^{13}C NMR

($CDCl_3$) δ 159.9, 144.35, 132.2, 128.4, 126.9, 125.77, 125.73, 125.7, 125.6, 123.1, 114.5, 55.4. Anal Calcd for $C_{14}H_{11}F_3O$ C, 66.6; H, 4.4; found C, 66.7; H, 4.5.

3c: Oil; IR (neat) 2923, 2854, 2227, 1457 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.74–7.72 (d, *J = 8.0 Hz*, 2H), 7.47-7.45 (d, *J = 8.0 Hz*, 2H), 7.34-7.28 (m, 3H), 2.28 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 140.7, 137.2, 133.6, 132.7, 129.5, 128.6, 127.8, 127.6, 126.3, 115.7, 111.5, 21.7. Anal Calcd for $C_{14}H_{11}N$ C, 87.01; H, 5.74; found C, 86.96; H, 5.01.

3d: Oil; IR (neat) 3030, 2925, 1901, 1611, 1484, 1438, 1330, 1258 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.83 (s, 1H), 7.79-7.74 (m, 1H), 7.62-7.48 (m, 4H), 7.29 (d, *J = 7.9* Hz, 2H), 2.42 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 141.9, 137.9, 131.1 (d, *J = 32.1* Hz), 130.2, 129.7, 129.2, 127.0, 124.2 (d, *J = 272.0* Hz), 123.6 (q, *J = 4.1 Hz*), 21.1. Anal Calcd for $C_{14}H_{11}F_3$ C, 71.18; H, 4.69; found C, 71.12; H, 4.63.

3e: m.p.: 104-105 °C; IR (neat) 3060, 2355, 1677, 1477, 748 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.29 (d, *J = 8.0 Hz*, 2H), 7.50 (d, *J = 8.0 Hz,* 2H), 7.35 (m, 3H), 7.22(d, *J = 7.2 Hz*, 1H), 2.27 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 148.3, 143.7, 140.2, 135.6,133.6, 129.5, 128.6, 126.8, 125.6, 124.8, 21.7. Anal Calcd for $C_{13}H_{11}NO_2$, C, 73.23; H, 5.20; found C, 73.25; H, 5.25 (Known compound, see: W. Han, C. Liu, and Z. Jina, *Adv. Synth. Catal*. 2008, **350**, 501).

3f: m.p.: 110.6-111.3 °C; IR (KBr) 1687, 1348, 821 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.22–8.21 (d, *J = 4.0 Hz*, 1H), 8.06-8.04 (d, *J = 8.0 Hz*, 2H), 7.77-7.67 (m, 3H), 7.45-7.43 (d, *J = 8.0 Hz*, 1H) 2.65 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 197.7, 149.6, 142.8, 135.6, 141.8, 138.8, 136.5, 134.3, 133.5, 133.4, 133.2, 133.1, 131.4, 129.2, 129.0, 127.4, 127.0, 123.1, 26.7, 20.2. Anal Calcd for $C_{15}H_{13}NO_3$, C, 70.58; H, 5.13; found C, 70.60; H, 5.17.

3g: m.p: 250.0-251.7 °C; IR (KBr) 2953, 2834, 2547, 1678, 1601, 1430, 1313, 1289 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.00–7.98 (d, *J = 7.7 Hz*, 2H), 7.76-7.68 (m, 4H), 7.07-7.05 (d, *J = 6.4 Hz*, 2H), 3.81 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 167.7, 160.07, 144.46, 131.76, 130.46, 129.37, 128.64, 126.62, 115.02, 55.73. Anal Calcd for $C_{14}H_{12}O_3$ C, 73.67; H,5.30; found C, 73.65; H,5.28

3h: m.p: 250.6 °C; IR (KBr) 2949, 2668, 2548, 1680 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.03–8.01 (d, *J = 8.0 Hz*, 2H), 7.81-7.75 (m, 4 H), 7.56-7.54 (d, *J = 8.0 Hz*, 2H); ^{13}C NMR (DMSO-d_6) δ 169.3,

141.6, 133.7, 132.8, 130.8, 129.4, 128.8, 127.2. Anal. Calcd. for $C_{13}H_9ClO_2$ C, 67.11; H, 3.90; found C, 67.06; H, 3.84.

3i: mp: 218.0-219.4 °C; IR (KBr) 2949, 2668, 2548, 1680, 1607, 1422, 1318, 1288 cm^{-1}; ^1H NMR (DMSO-d_6) δ 12.9 (s, 1H), 8.04-8.02 (d, *J = 8.0 Hz*, 2H), 7.81-7.79 (d, *J = 8.0 Hz*, 2H), 7.74-7.72 (d, *J = 8.0 Hz*, 2H), 7.75-7.48 (t, 2 H), 7.44-7.42 (d, *J = 8.0 Hz*, 2H); ^{13}C NMR (DMSO-d_6) δ 167.5, 144.7, 139.5, 130.4, 130.0, 129.5, 128.7, 127.4, 127.2. Anal Calcd for $C_{13}H_{10}O_2$ C, 78.77; H, 5.09 found C, 78.78; H, 5.10. (Known compound, see: K. M. Dawood, A. Kirschning *Tetrahedron* 2005, **61**, 12121).

3l: m.p: 97.9-98.4 °C; IR (KBr) 2923, 2360, 2341, 1673, 815 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.06–8.0 (d, *J = 8.0 Hz*, 2H), 7.68-7.66 (d, *J = 8 Hz*, 2H), 7.59-7.57 (d, *J = 8.0 Hz*, 2H), 7.47-7.45 (d, *J = 8.0 Hz*, 2H) 2.66 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 197.8, 141.2, 136.6, 135.1, 134.2, 129.4, 127.6, 29.3. Anal Calcd for $C_{14}H_{11}ClO$ C, 72.89; H, 4.81; found C, 72.85; H, 4.78

3m: m.p: 120.6-121.4 °C; IR (KBr) 1685, 1605, 1396, 1356, 1322, 823 cm^{-1}; ^1H NMR ($CDCl_3$) 8.09-8.07 (d, *J = 8.0 Hz* 2H), 7.75-7.70 (m, 6H), 2.67 (s, 3H); ^{13}C NMR ($CDCl_3$) δ197.6, 144.2, 143.4, 136.6, 129.1, 127.6, 127.5, 125.9, 26.73. Anal Calcd for $C_{15}H_{11}F_3O$ C, 68.19; H, 4.20 found. C, 68.20; H, 4.21. (Known compound, see: B. H. Lipshutz et al. *Org. Lett.,* 2008, **10**, 4279).

3n: mp: 153.3-154.5 °C; IR (KBr) 1673, 1600, 1294, 1818 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.03–8.01 (d, *J = 8.0 Hz*, 2 H), 7.67-7.65 (d, *J = 8.0 Hz*, 2 H), 7.61-7.58 (d, *J = 8.0 Hz,* 2 H), 7.03-7.01 (d, *J = 8.0 Hz* 2H), 3.88 (s, 3H), 2.64 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 197.7, 159.9, 145.4, 135.4, 132.3, 129.0, 128.4, 126.6, 114.4, 55.4, 26.6; Anal Calcd for $C_{11}H_{10}OS$ C, 69.44; H, 5.30; found C, 69.50; H, 5.35. (Known compound, see: C. C. Tzschucke et al, *Angew.Chem. Int. Ed.*. 2002, **41**, 4500).

3o: m.p.:152.7-153.9 °C; IR (KBr) 1683, 1594, 1508, 1338, 1263 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.34–8.32 (d, *J =8.0 Hz*, 2H), 8.11-8.09 (d, *J = 8.0 Hz*, 2H), 7.80-7.83 (d, *J =8.0 Hz*, 2H), 7.75-7.73 (d, *J =8.0 Hz*, 2H), 2.67 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 197.4, 147.7, 146.2,143.1, 137.2, 129.2, 128.1, 127.7, 124.3, 26.5. Anal Calcd for $C_{14}H_{11}NO_3$ C, 69.70; H, 4.60; found C, 69.73; H, 4.64.

3p: Oil °C; IR (neat) 3345, 3060, 1681, 1604, 1267 cm^{-1}; ^1H NMR (DMSO-d_6) δ 13.23 (s, 1H), 8.04–8.02 (d, *J = 8.0 Hz*, 1H), 7.99-7.92 (m, 3H), 7.73-7.71 (m, 1H), 7.50-7.25 (s,

1H), 7.23-7.21 (s, 1H) 2.60 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 198.6, 168.6, 140.9, 137.4, 134.7, 134.0, 132.8, 130.5, 128.6, 127.6, 127.4, 94.5, 27.3. Anal Calcd for $C_{15}H_{12}O_3$, C, 74.99; H, 5.03; found C, 74.97; H, 5.06.

3q: m.p.: 97.8-98.6 °C; IR (KBr) 2923, 2853, 1607, 1586, 1500, 1287, 1245, 806 cm^{-1}; ^{1}H NMR($CDCl_3$) δ 7.25-7.21 (m, 6H), 6.95 (d, *J = 8.8 Hz*, 2H), 3.85 (s, 3H), 2.27 (s, 3H); ^{13}C NMR (DMSO) δ 158.5, 141.5, 135.5, 134.4, 130.3, 130.2, 129.9, 126.9, 125.7, 113.5, 55.3, 20.5. Anal Calcd for; found. $C_{14}H_{14}O$ C, 84.81; H, 7.12; found C, 84.75; H, 7.07. (Known compound, see: Tao, Bin; *J. Org. Chem*, 2004*,* **69**, 4330).

3r: m.p.: 47.8-48.2: °C; IR (KBr) 3340, 1409, 1084 cm^{-1}; ^{1}H NMR (CDCl3) δ, 8.03 (d, *J = 8.5 Hz*, 2H), 7.69 (d, *J = 8.5 Hz*, 2H), 7.62–7.65 (m, 2H), 7.46–7.49 (m, 2H), 7.36–7.42 (m, 1H), 2.64 (s, 3H); ^{13}C NMR δ 198.1, 146.1, 140.2, 136.2, 129.3, 129.2, 128.6, 127.6, 127.5, 27.0; MS (m/e) 196 (M^+), 181, 152, 127, 102, 91, 76. Anal Calcd for; found. $C_{14}H_{14}O$ C, 84.81; H, 7.12; found C, 84.75; H, 7.07. (Known compound, see: G. E. Robinson, J. M. Vernon, J. Chem. Soc. C. 1971, 3363.).

Typical procedure for the one-pot chemoenzymatic synthesis of chiral biaryl alcohols

1-Chloro-4-iodobenzene (59.61 mg, 0.25 mmol), 0.05 mol% of Pd_{np}/Te-Dps, and 4-acetylphenylboronic acid (40,9 mg, 0.25 mmol.) in TRIS (2 mL, pH = 8.9, 100 mM) were orbitally stirred for 24 h at 100 °C with a Heidolph Synthesis System. After cooling the reaction to room temperature, 500 μL of 2-propanol, (*R*)-LB-ADH (200 U/mg, 414 μL), and $NADP^+$ (8.6 mg, 0.01mmol) were added and the mixture was stirred for 24h and at room temperature. After this time, the liquid phase was extracted with CH_2Cl_2, the organic layer was washed with deionized water and dried over Na_2SO_4. The solvent was removed under vacuum and the residue was purified by chromatography (SiO_2, 35 g, *n*-Hexane/AcOEt 70/30 v/v) to give 51.6 mg (89% yield) of

(*R*)-4a: m.p.:42.5-43.5 °C; IR (KBr) 3338, 1405, 1085, 898, 835 cm^{-1}; ^{1}H NMR (CDCl3) δ, 7.62-7.60 (d, *J = 8.5 Hz*, 2H), 7.46-7.41 (m, 5H), 4.97–4.93 (m, 1H), 1.72 (s, 1H), 1.56 (s, 3H); ^{13}C NMR δ 144.8, 140.8, 140.5, 128.7, 127.3, 127.1, 125.8, 70.2, 25.1. Anal Calcd for; found. $C_{14}H_{14}O$ C, 84.81; H, 7.12; found C, 84.77; H, 7.09. $[\alpha]_D^{20}$ +50 (0.1, $CHCl_3$), ee>99% ($[\alpha]_D^{28}$ -43.7 for the (*S*)-enantiomer, 99% ee; J.-i. Ito, S. Ujiie, and H. Nishiyama *Organometallics* 2009, ***28,*** 630.)

(*R*)-4b: m.p: 97.5-96.5 °C; IR (KBr) 3313, 2973, 1486, 1390, 815 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.58–7.52 (m, 4H), 7.48-7.46 (d, *J = 8.0 Hz*, 2H), 7.43-7.41 (d, *J = 8.0 Hz*, 2H), 4.98 (m, 1H), 1.84 (s, 1H), 1.52 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 145.4, 139.4, 139.2, 133.4, 128.9, 128.3, 127.1, 126.0,

70.1, 25.2 Anal Calcd for $C_{14}H_{13}ClO$ C, 72.89; H, 4.81; found C, 72.84; H, 4.77. $[\alpha]_D^{20}$ +32 (0.1, $CHCl_3$), ee>99%

(*R*)-4c: m.p: 115.7-116.5 °C; IR (KBr) 3369, 2979, 2360, 1396, 1128, 823 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.71 (s, 4H), 7.62-7.60 (d, *J = 8.0 Hz*, 2H), 7.51-7.49 (d, *J = 8.0 Hz*, 2H), 5.00 (m, 1H), 2.08 (s, 1H), 1.58-1.56 (d, *J = 8.0 Hz,* 3H); ^{13}C NMR ($CDCl_3$) δ 145.9, 144.4, 138.9, 129.5, 129.2, 127.4, 127.3, 126.1, 125.7, 125.6, 122.9, 70.0, 25.2 Anal Calcd for $C_{15}H_{13}F_3O$ C, 67.66; H, 4.92; found C, 67.60; H, 4.94. $[\alpha]_D^{20}$ +27 (0.1, $CHCl_3$), ee>99%.

Typical procedure for the determination of the enantiomeric excess of 4a and 4c

The enantiomeric excess was determined by enantioselective HPLC on the Chiralpak AS-H chiral stationary phase using the mixture *n*-hexane-2-propanol 95/5 (v/v) as a mobile phase. In these conditions, the (*R*)-enantiomers of **4a**-**4c** were eluted before the (*S*)-enantiomers. The enantiomeric peak identification was carried out by spiking racemic forms of biaryl alchohols with the enantiomers obtained from asymmetric reaction. The chromatographic data were: k_1 (*R*)-**4a** = 1.25, k_2 (*S*)-**4a** = 1.51, α = 1.21, Rs = 2.63; k_1 (*R*)-**4b** = 1.57, k_2 (*S*)-**4b** = 1.97, α = 1.25, Rs = 3.34; (*R*)-**4c** = 1.76, k_2 (*S*)-**4c** = 2.08, α = 1.18, Rs = 2.58. k_1: retention factor of the first eluted enantiomer, defined as $(t_1 - t_0)/t_0$ where t_0 is the void time of the column; α: enantioselectivity factor defined as k_2/k_1; Rs: resolution factor defined as $2(t_2-t_1)/(w_1+w_2)$ where t_1 and t_2 are retention times and w_1 and w_2 are band widths at the baseline in time units. Other analytical chromatographic conditions: flow-rate, 1.0 mL/min; temperature, 25 °C; detector: UV at 254 nm.

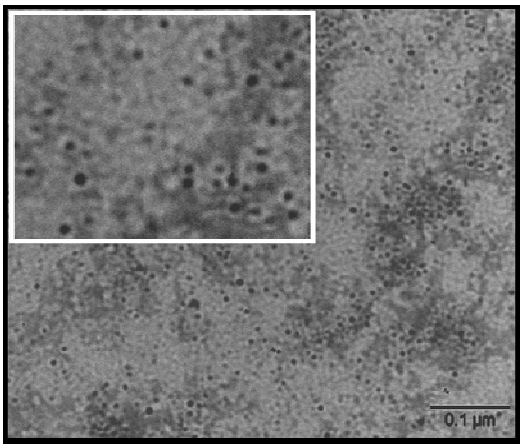

Figure S1. TEM image of Pd_{np}/Te-Dps negatively stained with PTA and 2x magnification in the inset.

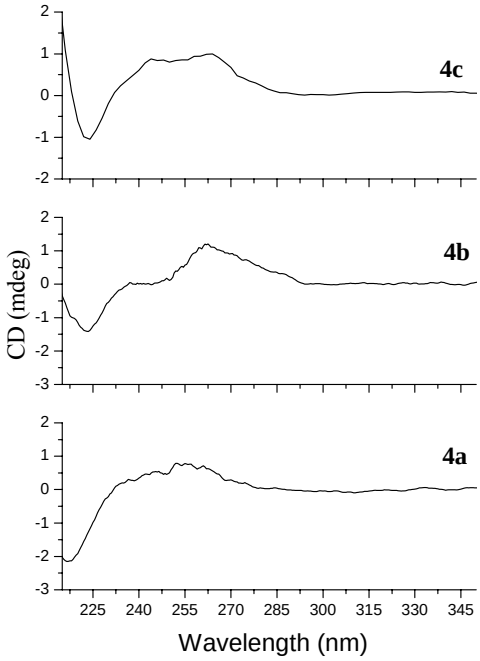

Figure S2. CD spectra of **4a**-**4c** in ethanol