Supporting Information

Rational Synthesis of Ultrasmall Palladium-Based Alloys with Superhydrophilicity as Biocompatible Agents and Recyclable Catalysts

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Experimental Procedures

1. Materials

Palladium Chloride($PdCl_2$, 90%), iron chloride (III) hexahydrate($FeCl_3 \cdot 6H_2O$), cobalt chloride hexahydrate($CoCl_2 \cdot 6H_2O$), cupric chloride dehydrate ($CuCl_2 \cdot 2H_2O$), Glutathione (GSH, Reduced 99%), Cysteine (Cys, 98%), rhodamine B (RhB, 95%), 4-Nitrophenol in methanol(4-NP, 99.5%), sodium hydroxide (NaOH, 95%), isopropanol (95%), methanol (95%), sodium borohydride ($NaBH_4$, 95%). All drugs are purchased from Aladdin. All chemicals were used without further purification.

2. Preparation of Cys- and GSH-capped palladium based biocatalysts (FePd. FePdCo, and PdCoCu NCs)

LaMer's nucleation and growth model suggests that a lower surfactant concentration would result in slower nucleation rate and a fewer number of nuclei so that larger nanoparticles can be generated. Thus, we intended to synthesize ultrasmall size Cys- and GSH capped biocatalysts using excessive amount of surfactant to increase catalytic efficiency while increasing the production rate of biocatalysts by adjusting the pH.

2.1 Ultra-small palladium based ternary Cys-/GSH-PdCoCu and FePdCo biocatalysts were synthesized via a one-pot chemical reduction strategy.

Briefly, $CoCl_2$ (31.2 mg, 0.131 mmol), $PdCl_2$ (23.2 mg, 0.130 mmol), $CuCl_2$ (22.4mg, 0.131mmol), and either cysteine (160 mg, 1.320 mmol) or GSH (160 mg, 0.520 mmol) was dissolved as a stabilizer. Then, after vigorously stirring for 10 minutes and N_2 treated about 0.5hour and the pH of the solution was adjusted to 8.5. Then, 4mL of aqueous $NaBH_4$ (1.0 MM) was rapidly added with vigorous stirring to produce Cys-PdCoCu or GSH-PdCoCu biocatalysts, respectively. The formation of the biocatalysts was indicated by the change in the color. The reaction solution was subsequently stirred for a further 3 h. Finally, isopropanol(10ml) was added to the prepared solution and stirred uniformly, and then centrifuged in a centrifuge for 20 minutes to precipitate the final product. The obtained GSH- or Cys-capped PdCoCu biocatalysts was Freeze dried for further physical and optical characterization. In addition, $CoCl_2$ (31.2 mg, 0.131 mmol), $PdCl_2$ (23.2 mg, 0.130 mmol), $FeCl_3.6H_2O$ (35mg, 0.205mmol), and either cysteine (160 mg, 1.320 mmol) or GSH (160 mg, 0.520 mmol) was dissolved as a stabilizer. It was used for forming Cys-FePdCo and GSH-FePdCo NCs, repectively.

2.2 Ultra-small palladium based bimetallic Cys-/ GSH-PdFe biocatalysts synthesis.

By using same approaches while synthesis Cys-/ GSH-FePd NCs via a one-pot chemical reduction strategy. Additionally, $PdCl_2$ (23.2 mg, 0.130 mmol), $FeCl_3$ (35mg, 0.205mmol), and either cysteine (160 mg, 1.320 mmol) or GSH (160 mg, 0.520 mmol) was dissolved as a stabilizer. Method steps are the same as above.

3. Characterization of optical properties (UV-vis, PL, FT-IR), zeta potential, tranmission electron microscopy(TEM), X-ray diffraction (XRD), thermogravimetric analysis(TGA).

UV-vis absorption spectra were recorded with a Lambda 25 UV-vis spectrophotometer (Perkin Elmer). The PL spectra were recorded by a LF-1204009 fluorescence spectrophotometer (Thermo Fisher Scientific, South

Korea). All the optical measurements were performed at room temperature. The size and ζ- potentials measurements of each sample was characterized by Mastersizer 2000 Laser Particle Size Analyzer (Malvern, British). In addition, the transmission electron microscopy (TEM) images of the samples were taken on a JEOL JEM-2100 transmission electron microscope with an acceleration voltage of 200 kV (carbon-coated copper grid was dipped in the sample solution to deposit it on the film).The typical weight loss of the sample was determined by thermogravimetric analysis (TGA, Mettler, Switzerland). Crystalline patterns of the materials were identified by powder X-ray diffraction (PXRD) using Cu Kα radiation (λ = 1.5418 Å) (SmartLab 9KW, Japan). The meanzeta potential of the prepared nanoparticles was detected by a Laser Particle Size Analyzer System (Malvern ZEN3600).

4. Cytotoxicity Assay in vitro

To explore the cytotoxicity of the Cys- and GSH-capped palladium based biocatalysts at different concentrations, L929 cells were cultured in 96-well plates at a density of 5.0×10^3 cells per well for 24 h. Culture medium containing two types Cys and GSH-FePd, FePdCo and PdCoCu colloidal solutions of same concentrations (0.39 mg/mL)was added to replace the original medium in the 96-well plate. These cells were cultured continuously for an additional 1, 2 and 3days. Then, the cell viability was evaluated via a Cell Counting Kit 8 (CCK8) following the manufacturer's instruction.[1,2] Absorbance (A450) was measured with a multimode plate reader (ELX800, BioTec, America). Thecell viability by relative growth rate (RGR) was calculated according to Equation 1:

$$RGR = OD_{sample} / OD_{blank} \times 100\% \quad (1)$$

To further quantify cytotoxicity, a live/dead cell staining experiment was also carried out. Then, the L929 cells were cultured with Cys-/ GSH- capped FePd, FePdCo and PdCoCu in culture medium for 3 days.The staining procedure and subsequent visualization were similar to the live staining experiment, which was outlined before.

5. Catalytic reaction and analysis of the intracellular reactive oxygen species levels

The reduction of RhB and 4-NP by $NaBH_4$ was selected as a model reaction to investigate the catalytic activity of palladium based biocatalysts following previously reported method.[2] Briefly, the reducing ability of the biocatalysts is dependent on its concentration, which was carefully monitored by checking the optical absorption at 553 nm. All kinetic assays were performed in path length (l) of 1.0 cm cuvettes. After adding the substrates (RhB and 4-NP) into each colloidal solution of Cys- and GSH- capped biocatalysts, the absorbance peak value at 553 nm and 400 nm, respectively. The calculation method was realized during absorption decrease and degradation approach, since the concentration of $NaBH_4$ is in excess, a pseudo-first order equation can be adapted for the analysis of the reaction kinetics Equation 2, where k is the first rate constant.[3]

$$C_t/C_0 = k_t \quad (2)$$

Catalyst recycle procedure: the alloys were collected by high-speed-centrifugation after the degradation process finish. Keep them under vacuum-drying conditions, and preparing the colloidal solution for next cycle utilization.

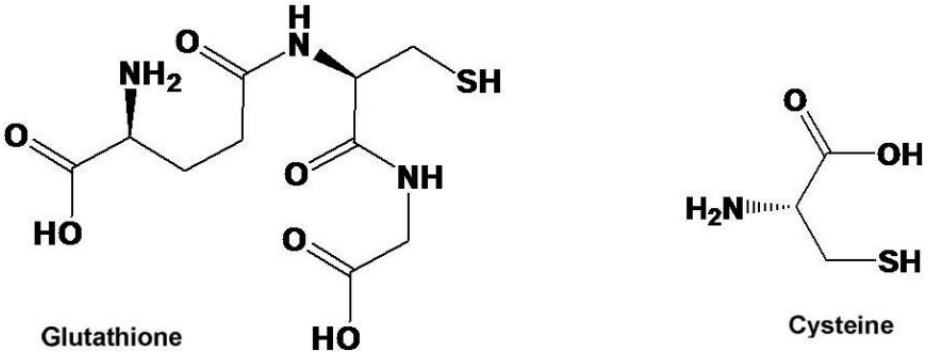

Figure S1: Glutathione (GSH) and Cysteine (Cys) molecular structures.

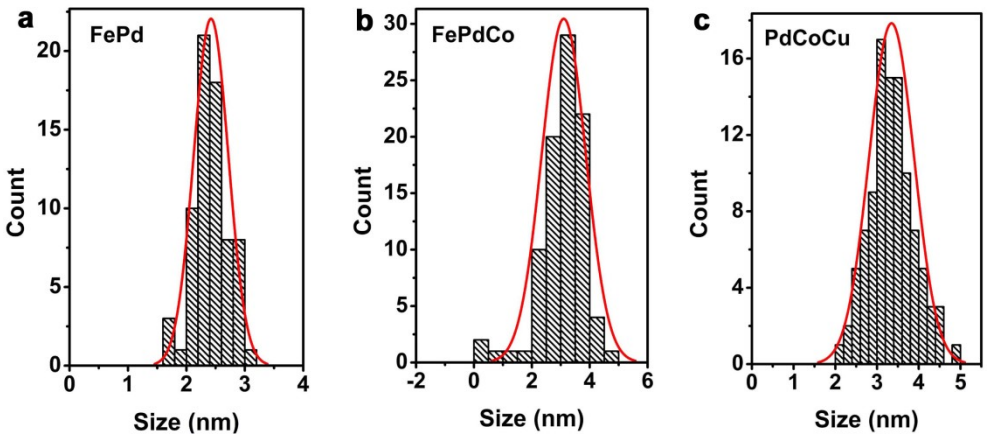

Figure S2: Size distribution of Glutathione (GSH) capped biocatalysts, (a)FePd, (b) FePdCo and (c) PdCoCu, respectively.

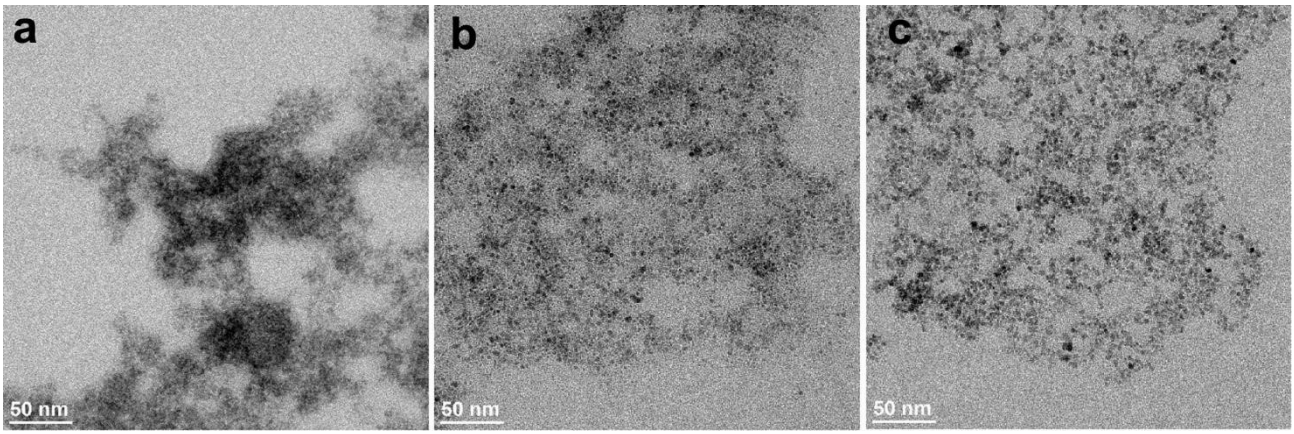

Figure S3: Low magnification of transmission electron microscope (TEM) images of GSH-capped (a)FePd, (b)FePdCo, and PdCoCu biocatalysts, respectively(insert scale bar is50 nm).

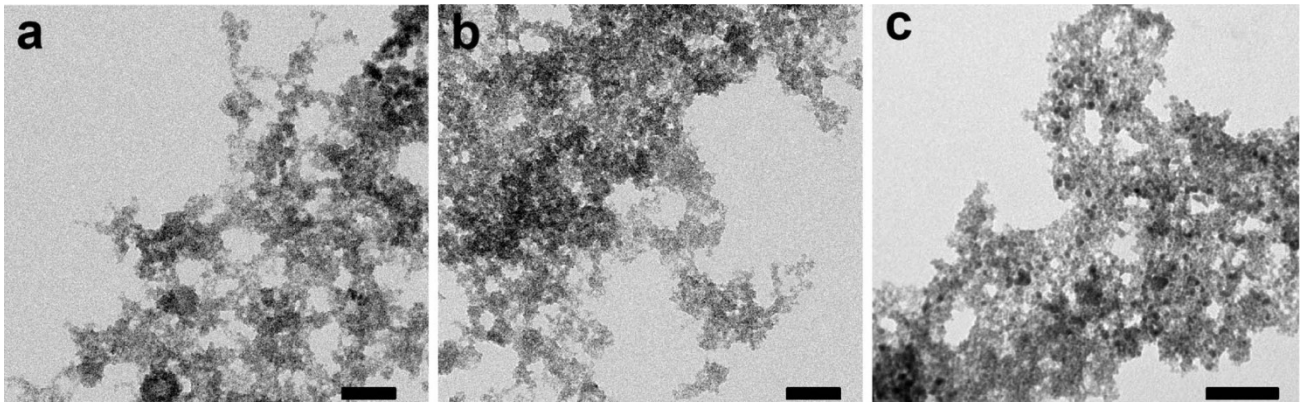

Figure S4: Low magnification of transmission electron microscope (TEM) images of Cys-capped (a)FePd, (b)FePdCo, and PdCoCu biocatalysts, respectively(insert scale bar is100 nm).

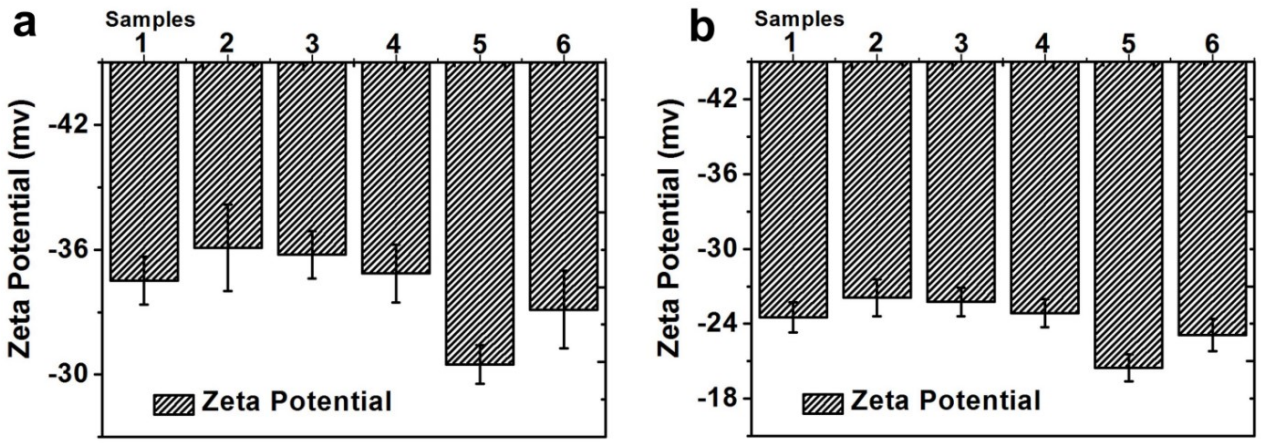

Figure S5: Zeta potential curves of as-synthesized GSH- and Cys- capped PdFe, PdCoFe and PdCoCu biocatalysts, which were incubated in water medium (a) and PBS (b), respectively (Sample1-6 (Cys-FePd, GSH-FePd, Cys-FePdCo, GSH-FePdCo, Cys-PdCoCu, and GSH-PdCoCu).

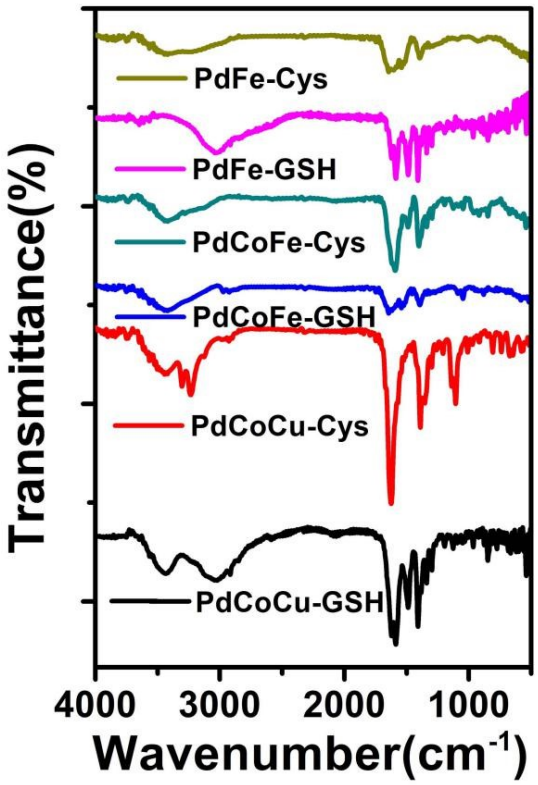

Figure S6: FT-IR spectra of as-synthesized GSH- and Cys- FePd, FePdCo and PdCoCu biocatalysts, respectively.

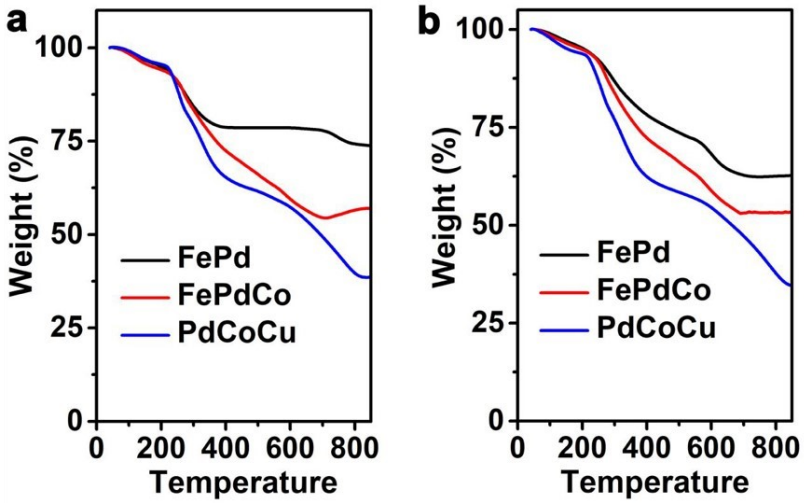

Figure S7: TGA of (a) GSH- and (b) Cys-capped FePd, FePdCo and PdCoCu biocatalysts, respectively.

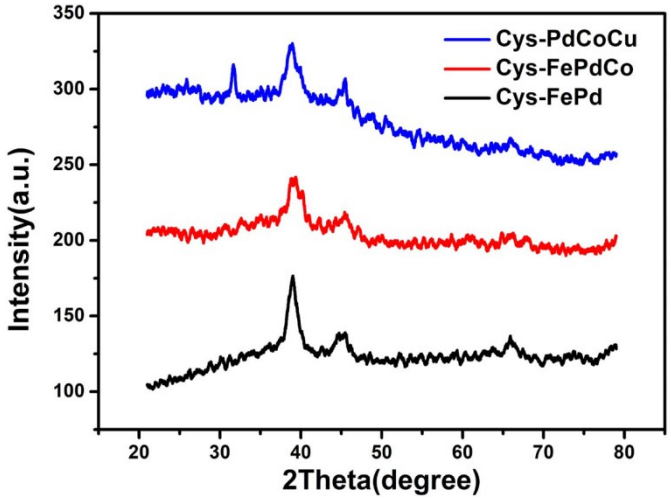

Figure S8: Powder XRD analysis of cysteine capped FePd, FePdCo and PdCoCu biocatalysts, respectively.

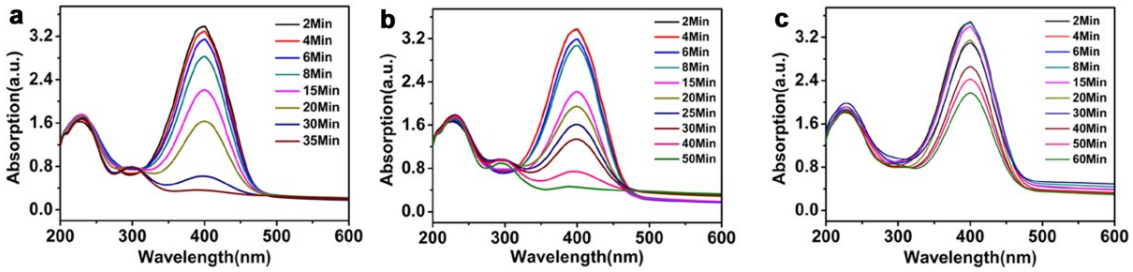

Figure S9: Catalytic properties of Cys capped biocatalysts. The UV-Vis spectra acquired during the reduction of 4-NP at different reaction intervals employing (a) FePd, (b) FePdCo and (c) PdCoCu as catalysts.

Figure S10: Catalytic properties of Cys capped biocatalysts. The UV-Vis spectra acquired during the reduction of RhB at different reaction intervals employing (a) FePd, (b) FePdCo and (c) PdCoCu as catalysts.

References

[1] E. Zhang, L. Zheng, J. Liu, B. Bai, C. Liu, *Materials science and Engineering C*, **2015**, *46*, 148-157.
[2] X. Xia, J. Zhang, N. Lu, M. J. Kim, K. Ghale, Y. Xu, E. McKenzie, J. Liu, H. Ye, *ACS Nano***2015**, *9*, 9994-10004.
[3] C. Singh, A. Goyal, S. Singhal,*Nanoscale*,**2015**, *6*, 7959-7970