Electronic Supplementary Information (ESI)

Photoenzymatic Synthesis through Sustainable NADH Regeneration by SiO_2-Supported Quantum Dots

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Experimental Methods

Materials: All chemicals, including tetraerhylorthosilicate (TEOS), ammonium hydroxide (NH_4OH), cadmium sulfoxide ($CdSO_4$), sodium sulfide (Na_2S), triethanolamine (TEOA), NAD^+, and glutamate dehydrogenase (GDH) were purchased from Sigma-Aldrich (St. Louis, MO) in reagent grade purity and were used without further purification. The organometallic mediator (**M**), [Cp*Rh(bpy)Cl]Cl, was synthesized according to the Kölle and Grätzel method (Kolle et al., 1989; Steckhan et al., 1991; Hollmann et al., 2003). SiO_2 beads were synthesized using the Stöber method (Stöber et al., 1968). Briefly, 100 mL of ethanol solution containing TEOS (0.5 mL) was mixed with a solution containing ammonium hydroxide dissolved in H_2O (NH_4OH, 5 N, 40 mL), and vigorously stirred for 2 h to ensure complete reaction. The procedure was repeated twice to produce larger particles, and the final product was stored in a refrigerator.

CdS nanocrystal growth on SiO_2 beads: CdS was deposited on the surface of SiO_2 beads through successive ionic layer adsorption reaction (called SILAR) (Baker and Kamat, 2009). Solutions containing cationic (Cd^{2+}) and anionic (S^{2-}) components were prepared by dissolving $CdSO_4$ (100 mM) and Na_2S (100 mM), respectively, in deionized water. The colloidal solution (20 mL) of SiO_2 beads was filtered to remove the alcohol solvent and then immersed in the Cd^{2+} precursor solution for 5 min. Cd^{2+}-adsorbed beads were washed with deionized water 3 times, introduced to S^{2-} solution for 5 min to produce CdS-coated SiO_2 beads, and then washed again with deionized water. The treatment with both cationic and anionic precursor solutions was repeated to increase the number of CdS-coating.

GDH immobilization on SiO_2 beads: SiO_2 beads were treated with a piranha solution (70% H_2SO_4 and 30% H_2O_2) for more than 12 h and rinsed with deionized water. The beads were subsequently treated with a 3% solution of APTS in ethanol/water (95:5 v/v) for 1 h. The aminopropylated SiO_2 bead was dispersed in 100% ethanol and cured at 110 °C for 1 h. After cooling at room temperature and washing with 95% ethanol, the bead surface was activated with N-hydroxysuccinimide ester (NHS) by incubating the beads for 3 h at room temperature in a DSC solution (20 mM) prepared in a sodium bicarbonate buffer (50 mM, pH 8.5). After incubation, the beads were washed with deionized water and dried under atmospheric conditions. NHS-activated SiO_2 particles (30 mg) were dispersed in a phosphate buffer (0.1 M, pH 7.0) containing 2 mg of GDH. The solution was stirred for 24 h at room temperature. Particles were collected by centrifugation and were rigorously washed with a fresh phosphate buffer until no protein could be detected in the supernatant solution. The Bradford assay was used to determine the protein content in the washing buffer. For repeated usability test, photocatalyst beads were collected by centrifugation and washed with deionized water before re-use. We attribute the slight decrease in activity (in Figure 2) after 4 rounds of repeated use to the loss of photocatalyst by sampling at every 10 minutes. According to our calculation, about 20% of photocatalyst can be lost by the sampling during 4 rounds of repeated use. Note that the concentration of photocatalyst is critical for light-driven cofactor regeneration according to our result (Figure S4). The slight increase in activity during 1st and 2nd use should be caused by different conditions of starting reaction; at 1st run, photocatalyst particles were completely dry and coagulated.

Photochemical reactions: The photochemical regeneration of NADH was performed in a quartz reactor (3 mL) under an argon atmosphere at room temperature. A 450-watt Xe-lamp (Oriel Co., USA) equipped with a 420 nm cut-off filter was used as a light source. Photo-regeneration of NADH was carried out by light illumination in a quartz reactor containing 1 mM NAD^+, 0.25 mM **M**, 15 wt% TEOA, and 100 mM phosphate buffer (pH 7.5) with SiO_2-CdS (1 mg/mL). For the enzymatic photosynthesis of L-glutamate, we conducted the reaction with NAD^+ (2 mM), SiO_2-CdS (1 mg/mL), **M** (500 μM), α-ketoglutarate (5 mM), ammonium sulfate (100 mM), and

GDH-SiO_2 (2 mg), which were prepared in a 0.1 M phosphate buffer (pH 7.5) containing 15w/v% TEOA.

Analysis: Spectrophotometric and spectrofluorometic analyses were performed by using a V-650 spectrophotometer (JASCO Co., Japan) and RF-5301PC spectrofluorometer (Shimadzu Co., Japan), respectively. Fluorescence spectra were obtained with an excitation at 250 nm. The concentration of NADH was measured by recording the absorbance at 340 nm. High performance liquid chromatography (LC-20A prominence, Shimadzu Co.) equipped with an Inertsil C_{18} column (ODS-3V, length, 150mm) was used for the analysis of L-glutamate synthesis by GDH. α-Ketoglutarate and L-glutamate were detected at 214 nm by eluting 0.05% phosphoric acid solution at the flow rate of 1.0 mL/min.

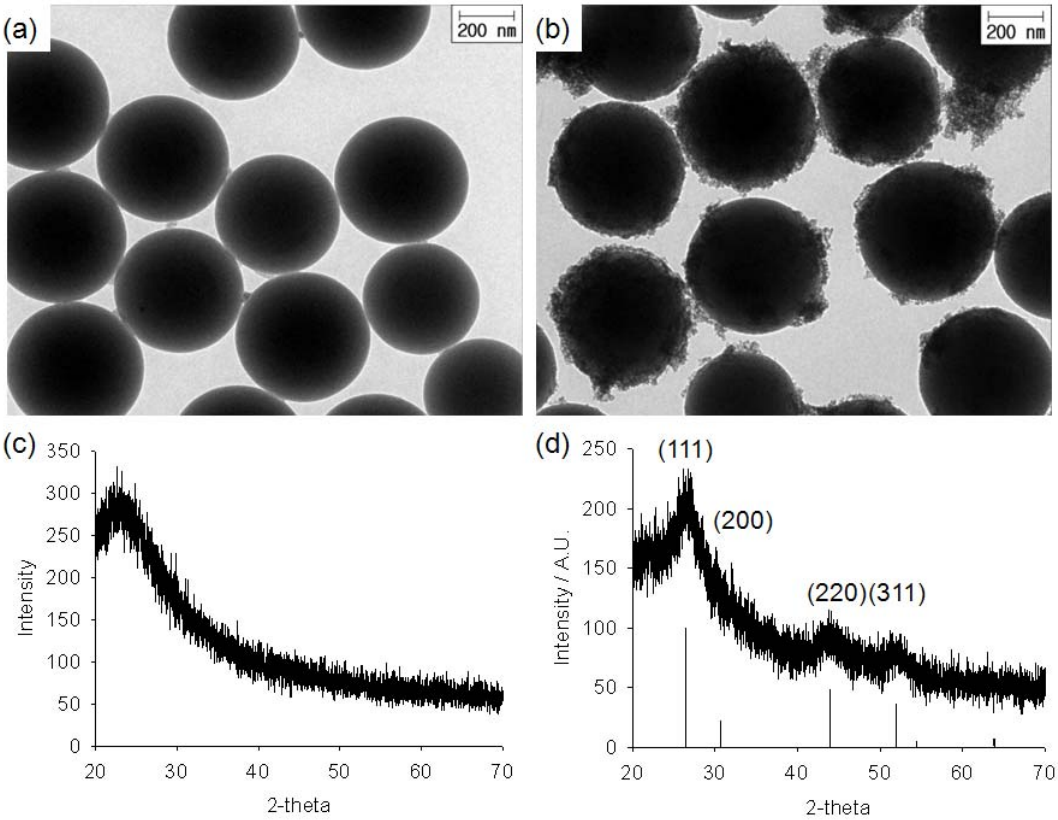

Figure S1. TEM images and X-ray diffraction (XRD) patterns of bare SiO_2 beads (a, c) and CdS (3x)-coated SiO_2 beads (b, d). The average size of the SiO_2 beads was 488.8±34.8 nm. The SiO_2-CdS sample exhibited a diffraction pattern of cubic crystal structure of CdS, while no diffraction peaks were observed in the bare SiO_2 sample.

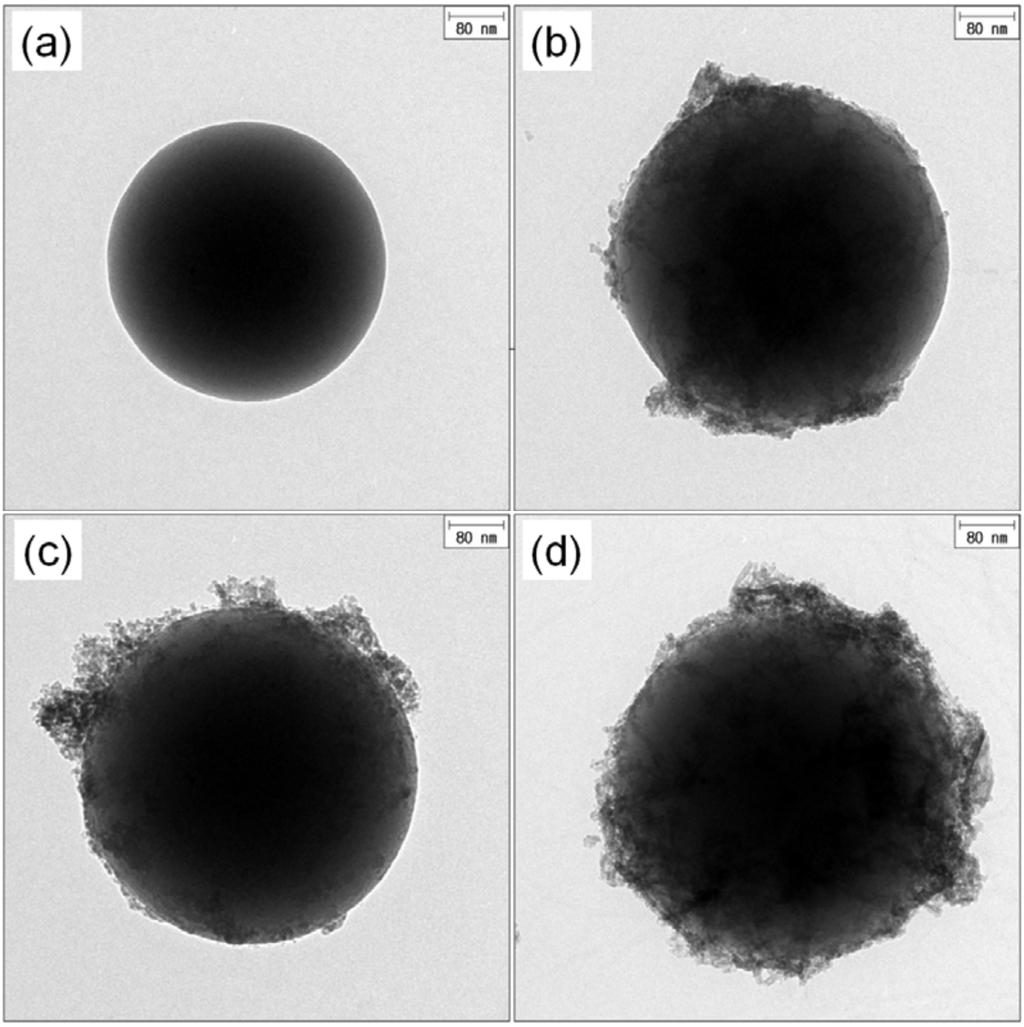

Figure S2. TEM images of SiO_2 beads with and without CdS coatings. (a) Bare SiO_2 bead and SiO_2 bead with (b) 1x, (c) 2x, (d) 3x CdS coating. The amount of CdS nanocrystals on the surface increased with the number of coatings. The size of CdS nanocrystals on the beads ranged from 3 to 7 nm.

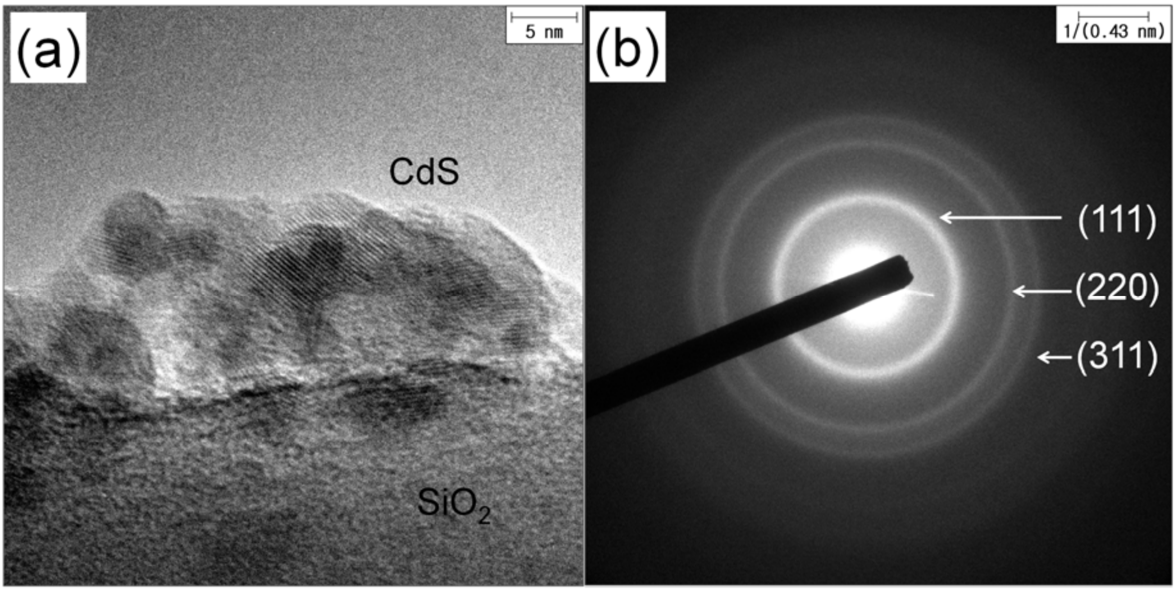

Figure S3. (a) TEM image and (b) selected area electron diffraction (SAED) pattern of SiO_2 bead with CdS-coating. Crystalline property of cubic CdS was confirmed by lattices in TEM image and the diffraction pattern.

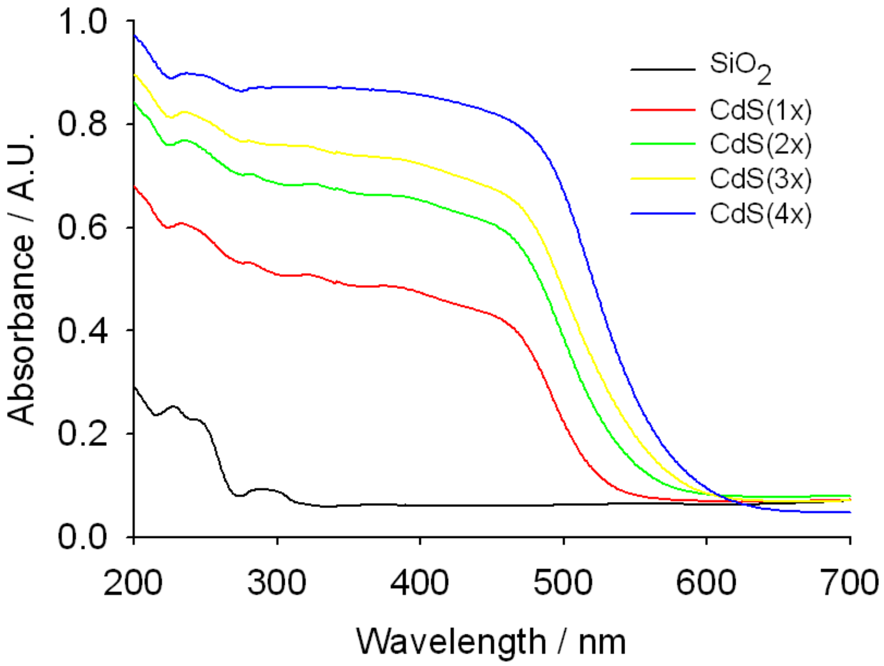

Figure S4. Diffuse reflectance spectra (DRS) of bare SiO_2 and SiO_2-CdS beads. With additional CdS coatings, the absorption intensity significantly increased in the visible light range, while bare SiO_2 exhibited no absorbance.

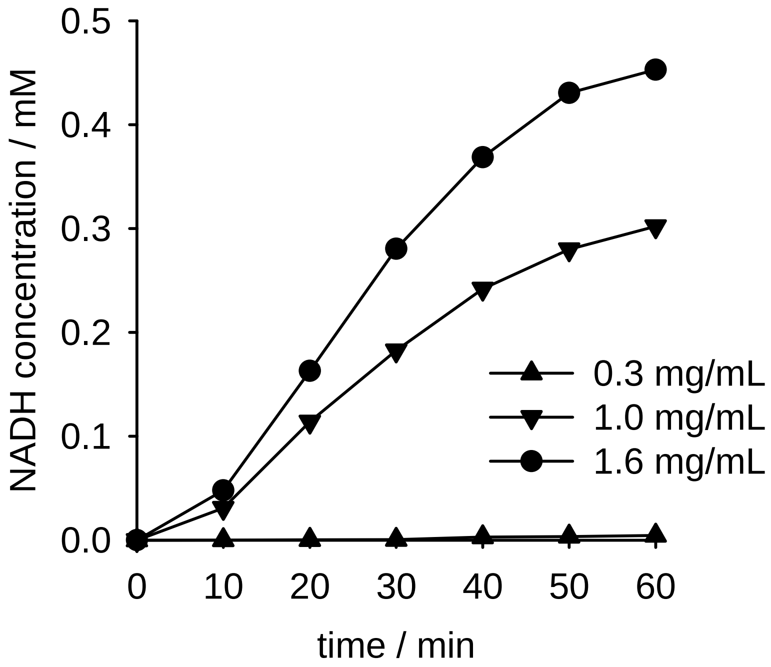

Figure S5. Photochemical NADH regeneration with different concentration of CdS-SiO_2 photocatalyst. The reaction was conducted with 0.3 ~ 1.6 mg/mL CdS-SiO_2, 1 mM NAD^+, 0.25 mM $[Cp^*Rh(bpy)H_2O]^{2+}$, and 15 wt% TEOA in 100 mM phosphate buffer (pH 7.5). The reaction rate was highly dependent on the concentration of photocatalyst.

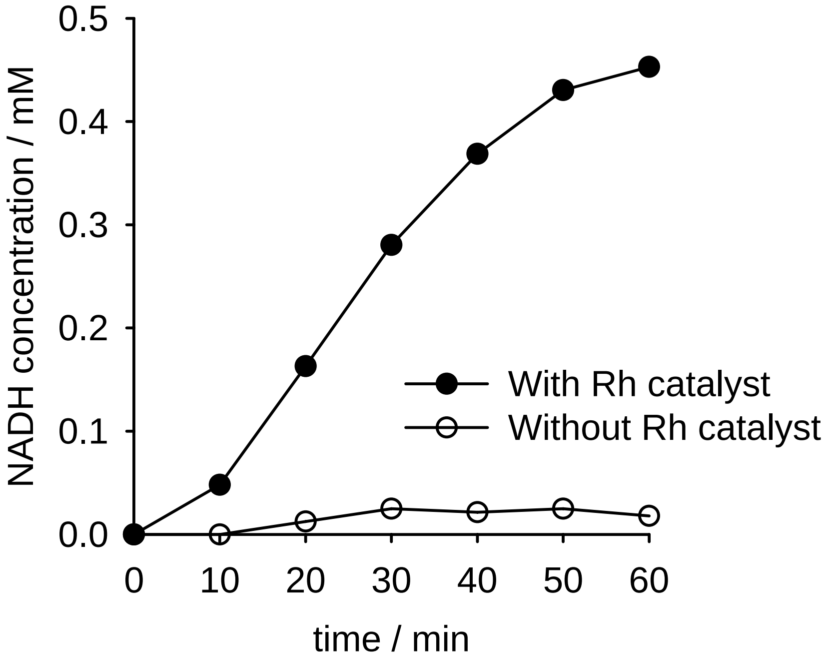

Figure S6. Photochemical NADH regeneration in the absence or presence of [Cp*Rh(bpy)H_2O]$^{2+}$. The reaction was conducted with 1.6 mg/mL CdS-SiO_2, 1 mM NAD$^+$, 0.25 mM [Cp*Rh(bpy)H_2O]$^{2+}$, and 15 wt% TEOA with 100 mM phosphate buffer (pH 7.5). Maximum 2.5% conversion was observed from the control experiment without [Cp*Rh(bpy)H_2O]$^{2+}$, while [Cp*Rh(bpy)H_2O]$^{2+}$ catalyzed reaction showed continuous increase in regenerated NADH concentration. The rate of NADH-formation by direct electron transfer from CdS-SiO_2 to NAD$^+$ was very slow compared to mediator-catalyzed reaction.

References

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