Photoenzymatic synthesis through sustainable NADH regeneration by SiO2-supported quantum dots.
Sustainable photochemical NADH regeneration and redox-enzymatic synthesis are accomplished by using CdS nanocrystals grown on the surface of SiO2 beads. CdS nanocrystals grown on SiO2 beads worked efficiently as a visible-light absorbing photocatalyst for in situ NADH regeneration with high catalytic activity and minimal loss of activity despite repeated uses.

Redox enzymes (or oxidoreductases) can catalyze industrially important synthetic reactions where conventional chemical catalysts fail. In many redox enzymatic reactions, it is critical to provide nicotinamide cofactors [i.e., NAD(P)H] as a stoichiometric equivalent. Due to the high cost of cofactors, numerous efforts have been made over recent decades for their in situ regeneration.1,2 The conventional approach of using secondary enzymes or whole cells to convert oxidized cofactors into a reduced form has been extensively studied with industrial scale applications.1,2 However, demands on efficient cofactor regeneration still exist and researchers are encouraged to pioneer nonconventional routes.3 Electrochemical regeneration of cofactors is considered as an attractive method because it uses cheap and mass-free matter (i.e., electrons) for cofactor regeneration leaving no byproduct. But direct electron transfer from electrodes to cofactor causes the generation of cofactor isomers and dimers, which are not applicable to enzymatic reactions and can even act as inhibitors. While the use of mediators may enhance the efficiency of electrochemical cofactor regeneration,4,6 inherent problems remain, such as electrode fouling and scale-up.

Nature hints at another route for cofactor regeneration through coupling photochemical transduction with biocatalytic reactions. The utilization of solar energy in green plants is accomplished by light-harvesting photosystems that absorb the clean and abundant solar energy in the visible light range and produce high energy electrons, which are stored in a reduced form of nicotinamide cofactors.7,8 The reduced cofactors are then used as an energy source for the production of carbohydrates through a cascade of multiple enzymatic reactions that take place in the Calvin cycle, followed by the regeneration of cofactors in the light reaction.9 Visible light-driven regeneration of cofactors has been attempted using organic/organometallic photocatalysts10-12 or inorganic photocatalysts.13-15 Recently, we reported on the application of quantum dots to the photochemical regeneration of nicotinamide cofactors in the visible light range.16 Note that inorganic photocatalysts, such as TiO2 and their variants, were inefficient when applied, especially under visible light. Quantum dots such as CdS, CdSe, and CdTe nanocrystals are attractive light-harvesting materials because their bandgaps are suitable for absorbing visible light while possessing robust photostability. The superior optical and photochemical properties of quantum dots enabled wide applications, such as solar electricity production (i.e., quantum dot-based solar cells), fluorescent imaging agents, and biosensors.17-19

Few studies of the stability and reusability of photochemical cofactor regeneration systems have been undertaken thus far despite its importance. Since cofactors are consumed by oxidoreductases in situ, a cofactor regeneration reaction should be stable and re-usable to sustain the enzymatic conversion. Thus, continuous regeneration of cofactors and the immobilization of enzymes are important issues for the development of sustainable biocatalytic processes since this regeneration can provide enhanced stability, repeated usability, and easy separation.20,21 When driven by light energy, high photostability of catalysts is critical for sustainable regeneration of cofactors. Natural photosensitizing pigments, such as chlorophylls, are easily bleached by light illumination, thus losing their visible-light-absorbing property.11

Herein we first report on the development of quantum dot-coated silica beads as a retrievable and reusable photosensitizer for sustainable NADH regeneration and redox enzymatic synthesis under visible light. We synthesized colloidal, submicron-size silica beads by using the Stöber method,22 producing mono-dispersed spherical silica nanoparticles through simple hydrolysis and the nucleation reactions of an alcoholic silica precursor. CdS quantum dots were successfully grown on the surface of SiO2 beads by a successive ionic layer adsorption reaction (called SILAR) of CdSO4 and Na2S (Scheme 1a). A negatively charged silica surface enabled spontaneous chemical reaction of each reaction component (i.e., Cd2+ and S2−) on the surface of the SiO2 beads. As a result of the ionic adsorption reaction, particulate CdS was
multiple SILAR treatments (Fig. S3, ESI†) with a gradual increase in the intensity of absorbance with a wavelength of 420 nm. The literature26,27 that reported an emission of blue photocatalysts showed that CdS-coated SiO2 beads was simultaneously quenched by the addition of M, that is, a CdS nanocluster. The CdS nanoparticles tend to form agglomerates. X-Ray diffraction analysis confirms the cubic crystal structure of CdS particles whereas amorphous SiO2 exhibited no significant diffraction peaks (Fig. S1, ESI†). The coating of CdS on the SiO2 beads provides visible-light-absorbing property to the SiO2 beads, with a gradual increase in the intensity of absorbance with multiple SILAR treatments (Fig. S3, ESI†).

Using CdS-coated SiO2 beads as a photosensitizer, we performed photochemical NADH regeneration through the mediation of \([\text{CdS}^{2+}]^{-}\) or M that regenerates NAD(P)H in an 'enzymatically-active' form \([i.e., 1,4-\text{NAD(P)H}]\) and plays the role of ferredoxin–NADP+ reductase in the natural photosynthetic system.23–25 We observed an efficient energy transfer between CdS-coated SiO2 beads and M by using fluorescence spectroscopy (Fig. 1a and b). In accordance with the literature26,27 that reported an emission of blue photoluminescence from SiO2 beads under a UV light, a weak fluorescence peak at 380 nm appeared from the colloidal solution of SiO2 beads under the UV-light excitation (250 nm). The fluorescence intensity increased when using CdS-coated beads, indicating that the fluorescent energy of CdS is transferred to the SiO2 beads. The fluorescence from bare SiO2 and CdS-coated SiO2 beads was simultaneously quenched by the presence of M in the reaction solution (Fig. 1b). This implies that SiO2 and M interact through a fluorescent energy transfer relationship. The metal center (Rh(II)) of M has a binding affinity for hydroxyl or carboxyl groups according to the literature,4,24,26 thus, an ionic affinity between the surface hydroxyl group of SiO2 and the metal center of M may be responsible for the energy transfer between SiO2 beads and M. In the case of CdS-coated SiO2 beads, the photosensitized electrons from CdS should be transferred more efficiently to M.

We carried out visible light-driven NADH regeneration by using CdS-coated SiO2 beads under visible light. While no regeneration of NADH from its oxidized form \([i.e., \text{NAD}^+]\) was detected during the dark stage, a rapid formation of NADH was observed upon irradiation of visible light \((\lambda > 420 \text{ nm})\), and its rate was dependent on the number of CdS coatings on the SiO2 beads (Fig. 1c). The regeneration rate increased as the number of coatings increased from one to three, but a significant decrease was noted with further coatings. At six coatings, a null yield of NADH regeneration was observed (data not shown). We attribute the decrease in the cofactor regeneration rate to the formation of CdS agglomerates and larger particles. According to the literature,6,29 the quantum properties and catalytic activity of semiconductor nanoparticles diminish with the increasing size of nanoclusters due to increasing defects, such as grain boundaries, that can cause charge recombination.

We further evaluated the photocatalytic stability and repeated usability of CdS-coated SiO2 beads for NADH regeneration. For the stability test, beads were collected by centrifugation and washed with deionized water before re-use. According to our result (Fig. 2), no significant decrease in photocatalytic activity was noted during 4 rounds of repeated use, and a high yield of NADH regeneration was maintained during the tests. We attribute the lag phase for first 10 minutes to incomplete dispersion of photocatalyst particles at early stage.

We utilized silica beads as a host matrix for photosensitizers \((i.e., \text{CdS nanoclusters})\) and redox enzymes by immobilizing them on separate silica beads, as illustratively depicted in Scheme 1b. As a model redox enzyme, we used glutamate dehydrogenase (GDH), a NADH-dependent enzyme. The GDH was immobilized by functionalizing the surface of bare SiO2 beads using N-hydroxysuccinimidyl (NHS) ester. The system, which consisted of SiO2–CdS (Bead 1) and SiO2–GDH (Bead 2), was evaluated for the photoenzymatic synthesis of L-glutamate from 2-keto glutarate by switching the light and dark stages. At Stage 1, we introduced only Bead 1 to observe
the photochemical regeneration of NADH under visible light. During the light reaction, approx. 80% of the NAD$^+$ was converted into NADH within 1 hour (Fig. 3). After Stage 1, the reactor containing Bead 1 was blocked from the light source, and Bead 2 was introduced to allow the GDH attached on SiO$_2$ beads to perform further enzymatic synthesis under dark condition (Stage 2). We observed that the photo-regenerated NADH (1.6 mM) was nearly consumed by Bead 2, and a stoichiometric amount of t-glutamate (1.6 mM) was produced. In order to regenerate the exhausted NADH, the reactor that now contained both Bead 1 and Bead 2 was again illuminated with visible light so that the light and dark reactions could occur simultaneously (Stage 3). Concentrations of both NADH and t-glutamate gradually increased again with the irradiation of visible light and the enzymatic synthesis of t-glutamate was completed in 300 minutes with 100% conversion of α-ketoglutarate (initial concentration: 5 mM). The slower rate of NADH regeneration at Stage 3 compared with that at Stage 1 indicates that photo-regenerated NADH is partially consumed by the enzymatic reaction on Bead 2.

In summary, we have demonstrated that a SiO$_2$ beads-based platform can be successfully used for sustainable photoenzymatic synthesis. Photo- and bio-catalytic reaction components were separately immobilized on the surface of sub-micron colloidal SiO$_2$ beads. The platform for a continuous and efficient supply of in situ regenerated cofactors should promise a sustainable photobiosynthesis of fine chemicals.

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Notes and references