Electronic Supplementary Information

Artificial Photosynthesis on a Chip: Microfluidic Cofactor Regeneration and Photoenzymatic Synthesis under Visible Light

Joon Seok Lee, Sahng Ha Lee, Jae Hong Kim, and Chan Beum Park*

Department of Materials Science and Engineering, Korea Advanced Institute of Science and Technology, Daejeon, 305-701 Korea. *Email: parkcb@kaist.ac.kr

Experimental Section

Materials: All chemicals, including triethanolamine (TEOA), NAD⁺, α-ketoglutarate, L-glutamate dehydrogenase (GDH), were purchased from Sigma-Aldrich (St. Louis, MO). The carboxylated quantum dots (QD), Qdot®565 and Qdot®655, were purchased from Invitrogen Inc. (Carlsbad, CA). [Cp*Rh(bpy)H2O]²⁺ (M) was synthesized according to previous studies.[1,2]

Fabrication of Microfluidic Artificial Photosynthesis System: Microchannels were fabricated using soft lithography and rapid prototyping following the literatures.[3-5] A silicon master was fabricated by patterning a negative photoresist (SU-8, MicroChem, Newton, MA) using conventional photolithography. A positive replica with an embossed microchannel network was obtained by replica-molding of polydimethylsiloxane (PDM) (DowCorning Midland, MI) against the master. The microfluidic system is made of two layers: a top fluidic layer and a bottom valve layer (Figure S3). The valve channel with a dimension of 500 μm (width) x 100 μm (height) x 1200 μm (length) passes underneath the main fluidic channel having a dimension of 500 μm (width) x 100 μm (height) x 10 cm (length). Microchannels were formed by attaching two PDMS layers to a glass slide using an oxygen plasma treatment and bonded together to seal irreversibly. Holes that consist of inlets a, b, outlets a’, b’ and an air-inject part were punched for the interconnection of patterned PDMS microchannels (Figure S3). The glass slides were cleaned with piranha solution of H2SO4/H2O2 (7/3, v/v) for 15 min at 60 °C, rinsed with deionized water, and dried with N2 before bonding. The surface inside each microchannel was chemically activated for the covalent attachment of QD and enzyme. First, a 3% solution of 3-amino-propyltriethoxysilane (APTS) in ethanol/water (95:5 v/v) was injected at a flow rate of 5 μL/h into the microchannel for 1 h for aminopropylation using 11 Plus syringe pump (Harvard Apparatus, Holliston, MA). The microchannel was then washed with 100% ethanol and cured at 100 °C. 15 μl CdSe QD (8μM) solution including 3μl 1-
ethyl-3-(dimethylaminopropyl) carbodiimide hydrochloride (EDC, 2.2 μM in methanol) 3 μl
N-hydroxysulfosuccinimide (NHS, 4 μM in methanol), and 9 μl methanol, was injected into
the light-dependent reaction zone. During the injection of QD solution, the valve was closed
to block any leakage into the light-independent reaction zone. For the attachment of GDH in
the light-independent reaction zone, the microchannel was activated with NHS by the
injection of a 20 mM N,N'-disuccinimidylcarbonate (DSC) solution in a sodium bicarbonate
buffer (50 mM, pH 8.5) for 3 h at room temperature. After the reaction was completed, the
microchannel was washed with deionized water and dried. Finally, enzyme solution was
applied to the microchannel at a low flow rate for approximately 24 h. Then, the reactor
channel was washed with a phosphate buffer (10 mM, pH 7.4) and stored at 4 °C until use.

**Cofactor Photoregeneration and Enzymatic Synthesis of L-Glutamate:** Photochemical
regeneration of NADH cofactor was performed in the microfluidic system at room
temperature. A 400 W xenon lamp equipped with a 420 nm cut-off filter was used as a light
source (light intensity: 62.0 mW/cm²). The feeding solution was composed of NAD⁺ (1 mM),
M (0.25 mM), TEOA (15% w/v), and phosphate buffer (100 mM). The solution was injected
at various flow rates through the inlet a in Figure S3. The feeding solution for the
photosynthesis of L-glutamate was composed of M (0.5 mM), NAD⁺ (2 mM), α-ketoglutarate
(1 mM), and ammonium sulfate (100 mM), based on a phosphate buffer (100 mM), with
TEOA (15% w/v; pH 8.0). The solution flowing through outlet a' (light-dependent reaction)
or outlet b' (light-independent reaction) was collected in protein low-binding tubes (Protein
LoBind, Eppendorf, Hamburg, Germany), which were sealed to avoid any evaporation of the
solution.

**Characterization:** The morphology of CdSe QDs was investigated using a Tecnai F20 TEM
(Philips, Japan) operating at 200 kV. UV/Vis absorption spectra of QDs were measured using
a V-650 spectrophotometer (JASCO, Japan). Spectrofluorometric analysis of QDs at different
concentrations of M was performed using RF-5301PC (Shimadzu Co., Japan) with an
excitation wavelength of 400 nm. The fluorescence microscope (Eclipse 80i; Nikon, Japan)
was focused on the bottom surface of the microchannel to obtain uniform images of each
microchannel. The following filter sets were used (excitation, emission): 565-QDs (450-490,
505) and 655-QDs (510-560, 575), respectively. The immobilized CdSe QDs on the
microchannel surface was analyzed by measuring their XPS spectra using a JPS-9000MX
spectrometer (JEOL Ltd., Japan). The concentration of NADH collected from the outlet of the
microchannel was measured spectrophotometrically from its absorbance at 340 nm. High-performance liquid chromatography (LC-20A prominence, Shimadzu Co., Japan), equipped with an Inertsil C18 column (ODS-3V, length, 150 mm), was used for the analysis of enzymatic reactions in the microfluidic system. Samples were eluted by phosphoric acid (0.085%) at a flow rate of 1.0 ml/min and detected at 210 nm.

REFERENCES

**Fig. S1** Absorption (dot) and PL spectra (line) of 565-QDs and 655-QDs. The CdSe QDs have maximum emission at 565 and 655 nm, respectively. The absorption and emission spectra of 565 and 655-QDs indicate their broad range of visible light absorption and narrow emission spectrum.
**Fig. S2** HRTEM images of 565 and 655-CdSe QDs. The images revealed that QDs were uniform in size: approximately 4 x 4 nm for 565-QDs and 15 x 5 nm for 655-QDs. Each QD exhibits clear crystalline lattice of CdSe nanostructure. Inset: Selected area electron diffraction (SAED) pattern having diffraction rings that correspond to the cubic phase of CdSe.

**Fig. S3** A working principle of separate zone formation of the microfluidic platform. The platform is made of two layers: a top fluidic layer and a bottom valve layer. The valve channel passes underneath the fluidic channel. (A) The thin membrane between the valve channel and the flow channel functions as a cutter. (B) When we increased the valve pressure, the thin membrane between the fluidic channel and the valve was deflected separating the main flow for two reaction zones as a barrier. (C) When the pressure was released, the membrane was restored to its original shape and the main flow resumed.
Fig. S4 XPS narrow scan spectrum of Cd3d and Se3d on CdSe-immobilized microchannel. In the case of absorption of QDs on a bare microchannel surface, no peaks were observed after several washing, whereas in the case of covalently-attached QDs to the pretreated microchannel, two peaks of Cd3d\(3/2\) and Cd3d\(5/2\) are centered at 411.9 and 405.4 eV, respectively. The peak at 54.2 eV, observed in the Se energy region, is attributed to the Se3d transition.
**Fig. S5** Fluorescence-quenching of 655-CdSe QDs in the presence of M. The fluorescence of 655-CdSe gradually decreased with the increasing concentration of M from 0 to 250 μM. The fluorescence-quenching implies that photo-excited electrons of CdSe (electron donor) were transferred to M, an electron acceptor.

**Fig. S6** Photocatalytic activity of CdSe-immobilized microchannels during repeated uses. Photocatalytic activity of both 655-CdSe and 565-CdSe QDs was maintained during 5 times of recycling.