Supporting Information

DNA Release from a Modified Electrode Triggered by a Bioelectrocatalytic Process

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Experimental Details

1. Chemicals and Materials

Enzymes: α-amyloglucosidase from *Aspergillus niger* (AMG; EC 3.2.1.3) and glucose dehydrogenase from *Pseudomonas sp.* (GDH; E.C. 1.1.1.47) were purchased from MilliporeSigma (formerly Sigma-Aldrich); bilirubin oxidase from *Myrothecium verrucaria* (BOx; E.C. 1.3.3.5) was kindly donated by Amano Enzyme Inc., U.S.A. Single-stranded oligonucleotide with a fluorescent label, FAM-DNA, (6-FAM-5'-TGC AGA CGT TGA AGG ATC CTC-3') was purchased from Integrated DNA Technologies (Coralville, IA, USA) (FAM attached to the DNA is a fluorescein derivative; https://www.idtdna.com/site/Catalog/Modifications/Product/1108). (3-Aminopropyl)triethoxysilane (**1**), 4-carboxyphenylboronic acid (**2**), 1-methylpyridinium-3-carboxylate hydrochloride (trigonelline hydrochloride) (**3**), biotin (**4**), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC), *N*-hydroxysuccinimide (NHS), polyethylene imine (PEI, **5**, average M_w ca. 750,000), avidin, pyrroloquinoline quinone (PQQ), nicotinamide adenine dinucleotide (oxidized and reduced forms, NAD^+ and NADH), (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) (HEPES buffer), *tris*(hydroxymethyl)-aminomethane (TRIS buffer), pyrenebutanoic acid succinimidyl ester (PBSE), $K_3[Fe(CN)_6]$, $K_4[Fe(CN)_6]$, and other standard organic and inorganic materials and reactants were purchased from MilliporeSigma (formerly Sigma-Aldrich). The structures of **1-4**, EDC and NHS are shown in the main paper in Figure 1, the structure of **5** is shown in Figure 2, the structure of FAM label is shown in Figure 5, the structures of PQQ, NAD^+ and NADH are shown in Figure 6. SiO_2 nanoparticles (SiO_2-NPs; ca. 200 nm) were purchased from Fiber Optics Center Inc. All commercial chemicals were used as supplied without further purification. Ultrapure water (18.2 MΩ·cm) from a NANOpure Diamond (Barnstead) source was used in all of the experiments. Graphite pencil rods (Pentel-Super Hi-polymer lead 0.9 mm HB, Walmart) were used as conducting supports for preparing modified electrodes.

2. Preparation of the Modified SiO_2-NPs

SiO_2-NPs (100 mg) were dispersed in 1 mL H_2O that had been adjusted to pH 9.0 (Mettler Toledo Seven Easy pH Meter) using NaOH. The dispersion was sonicated (VWR Model 75T sonication bath) for 40 minutes until homogenous suspension was obtained, then it was centrifuged (Beckman Coulter Microfuge 22R Centrifuge) for 10 minutes at 10,000 rpm. The H_2O was replaced with ethanol three times, centrifuging the particles each time. The SiO_2-NPs in the ethanolic dispersion (1 mL) were reacted with 3-aminopropyl)triethoxysilane (3% v/v) for 3 hours under mild shaking (VWR Standard Analog Shaker). The silanized SiO_2-NPs were carefully rinsed 3 times with ethanol by centrifuging and replacing ethanol each time. Then, the ethanol was replaced with 25 mM HEPES buffer, pH 7.2, 3 times, centrifuging each time. Then, the silanized SiO_2-NPs dispersed in the HEPES buffer were reacted with a mixture of 4-carboxyphenylboronic acid (**2**, 24 mM), trigonelline (**3**, 6 mM) and biotin (**4**, 6 mM). Note that the molar ratio 4:1:1 of the reacting species was optimized experimentally. EDC (100 mM) and NHS (100 mM) were added to the reacting mixture to bind **2**, **3** and **4** to the SiO_2-NPs by carbodiimide coupling of their carboxylic groups to the amino groups of the silanized SiO_2-NPs. The reaction was conducted overnight under mild shaking. The modified SiO_2-NPs were centrifuged and washed with 25 mM HEPES buffer, pH 7.2, 3 times, then suspended in the HEPES buffer. All reaction steps were performed at room temperature (20 ± 2 °C). The reaction steps in preparation of the modified SiO_2-NPs are shown in the main paper in Figure 1.

3. Electrode Modification for the FAM-DNA Release

Graphite pencil rods (GPR) were used as the electrode material. A GPR electrode was polished with 1500 Grit sandpaper (3 M Wettodry), sonicated in ethanol for 3 minutes, and finally polished with a KimTech kimwipe (Kimberly-Clark Professional) to prepare the electrode surface for modification. The first modification step was physical adsorption of PEI (50 mg/mL in water) under mild shaking for 30 minutes, followed by rinsing with water. The adsorbed PEI provided amino groups at the electrode surface for the next modification step. Then, the electrode was left for 30 min to incubate in a glutaraldehyde aqueous solution (2.5% v/v), after that it was rinsed with water. Then, the electrode was left to incubate in a BOx solution (10 mg/mL in 10 mM TRIS

buffer, pH 7, with 100 mM Na_2SO_4) shaking for one hour, then washed with water. Next, the modified electrode was reacted with 6 mM biotin, 100 mM EDC and 100 mM NHS in 10 mM HEPES buffer, pH 7.5, under mild shaking for 3 hours, followed by rinsing with the HEPES buffer. Then, the biotin-modified electrode was reacted with avidin solution (50 μg/mL in water) under mild shaking for 1 hour, followed by rinsing with water. The biotin-avidin-functionalized electrode was reacted with the biotinylated modified SiO_2-NPS under stirring for 1 hour, then rinsed with the HEPES buffer. All reaction steps were performed at room temperature (20 ± 2 °C). The modified electrode geometrical area was ca. 0.3 cm^2. The reaction steps in preparation of the modified electrode are shown in the main paper in Figure 2.

4. FAM-DNA Loading on the Modified Electrode and its Electrochemically Stimulated Release

The modified GPR electrode prepared according to the procedure detailed above (see also the scheme in the main paper in Figure 2) was reacted with FAM-DNA (125 μL, 0.5 μM) under mild shaking for 1.5 hours. The modified electrode with the adsorbed FAM-DNA was rinsed four times with 3 mL 1 mM HEPES buffer, pH 7.5, (5 min, 30 min, 15 min and 10 min in the rinsing steps), each time with a new fresh solution. The desorption of the FAM-DNA in the rinsing steps was considered as the "leakage" of the weakly bound FAM-DNA. Then, the modified electrode was placed in an electrochemical cell, also containing a graphite counter electrode and a reference electrode in 3 mL of 1 mM HEPES buffer, pH 7.5, containing O_2 under equilibrium with air. The FAM-DNA release was stimulated by applying constant potential of 0 V (vs. Ag/AgCl reference) on the modified electrode for various time-intervals. The background electrolyte solution was analyzed for the presence of the released FAM-DNA by measuring fluorescence (Varian, Cary Eclipse fluorescence spectrophotometer) corresponding the FAM fluorescent label. The measurements were performed with an excitation wavelength of 485 nm and emission wavelength from 500 to 560 nm using a fluorescent spectrophotometer (Varian, Cary Eclipse). Control experiments were performed in the absence of O_2, when anaerobic conditions were achieved by purging the solution with argon.

5. Electrochemical Measurements

Electrochemical experiments, including constant potential electrolysis and cyclic voltammetry, were performed using an electrochemical workstation (ECO Chemie Autolab PASTAT 10) and GPES 4.9 (General Purpose Electrochemical System) software. All potentials were measured using a Metrohm Ag|AgCl|KCl, 3 M, reference electrode and a graphite slab was used as the counter electrode. Cyclic voltammetry measurements in the absence and presence of O_2 (under equilibrium with air) were performed with the modified electrode containing all deposited species excluding the adsorbed FAM-DNA. The background electrolyte was 1 mM HEPES buffer solution, pH 7.5. Faradaic impedance measurements were performed with the electrochemical analyzer using the software package FRA 4.9 (Frequency Response Analyzer) with the background electrolyte 1 mM HEPES buffer solution, pH 7.5, in the presence of 1 mM solution of $[Fe(CN)_6]^{3-/4-}$ (1:1 molar ration) redox probe. The impedance spectra in the form of Nyquist plots were recorded in the absence and presence of O_2, while applying a bias potential of 0 V and using a 10 mV alternative voltage in the frequency range 50 kHz – 0.01 Hz.

6. Fluorescent and SEM Imaging

The modified electrode with the FAM-DNA adsorbed and later released was analyzed using Leica TCS SP5 II Tandem Scanning Confocal Microscope with excitation wavelength of 485 nm (see Figure S1 for the calibration plot of the FAM-DNA fluorescence vs. FAM-DNA concentration). The observed green fluorescence from the electrode surface, Figure S2, was used to follow the FAM-DNA adsorption and then its release. Field emission scanning electron microscopy (SEM) was used to characterize the modified electrode with the bound SiO_2-NPs. The SEM imaging was conducted using FESEM JEOL-7400 electron microscope (JEOL USA, Inc.).

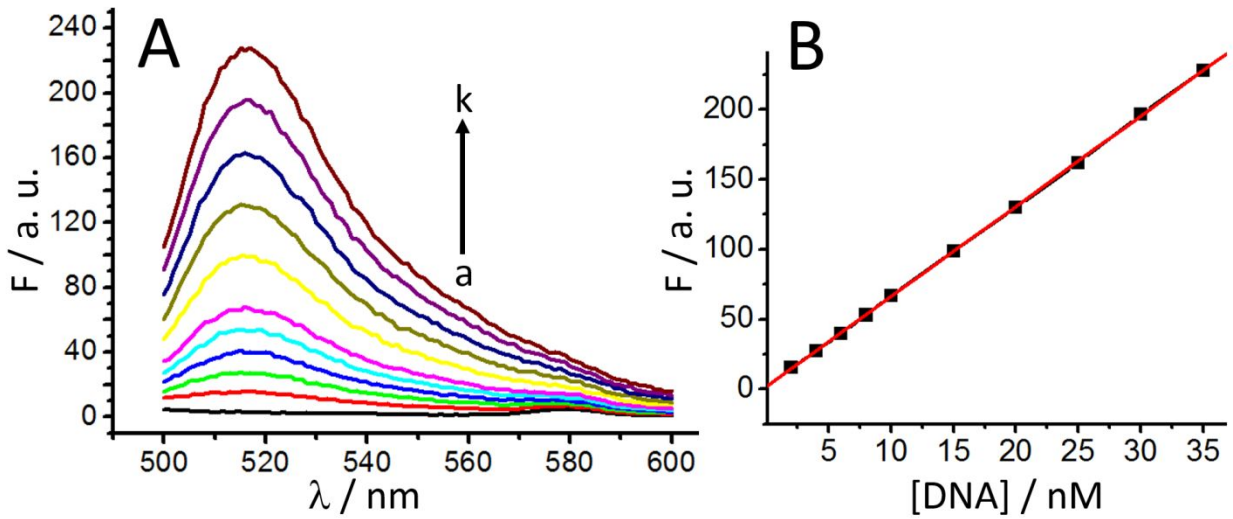

Figure S1. (A) Fluorescence spectra measured in a solution composed of 1 mM HEPES buffer, pH 7.5, containing 100 mM Na_2SO_4, containing different concentration of the dye-labelled DNA (5'-/56-FAM/TGC AGA CGT TGA AGG ATC CTC -3'): (a) base line (no DNA in the solution), (b) 2 nM, (c) 4 nM, (d) 6 nM, (e) 8 nM, (f) 10 nM, (g) 15 nM, (h) 20 nM, (i) 25 nM, (j) 30 nM, (k) 35 nM. (B) Calibration plot of the fluorescence intensity at λ_{max} = 517 nm vs. the concentration of DNA present in the analyzed solution. The fluorescence is expressed in arbitrary units measured by the fluorimeter. This calibration plot was used to calculate the amount of the DNA released from the modified electrode.

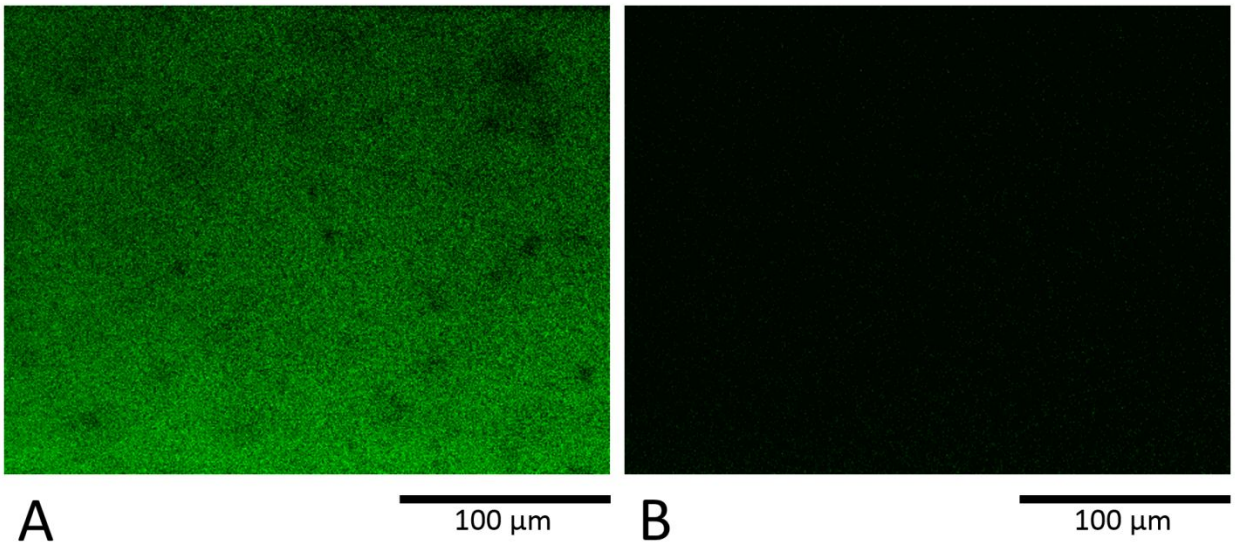

Figure S2. Confocal microscope images of the BOx/SiO_2-NPs-modified electrode surface with loaded FAM-DNA prior to the electrochemically stimulated release (A) and after the release was completed by applying 0 V for 1 hour in the presence of O_2 (under equilibrium with air) (B). The green color in the images corresponds to the fluorescence of FAM-DNA.

7. Thylakoid Membranes (TMs) Extraction

Spinach plants (*Spinacia oleracea*) were purchased from a local supermarket (Walmart). 45 g of spinach leaves were homogenized in a kitchen blender containing 100 mL of preparation medium containing 50 mM phosphate-buffered saline (pH 7.4), 5 mM $MgCl_2$ and 300 mM sucrose. The slurry was filtered through four layers of nylon mesh (20 µm) and centrifuged at 2000 × g for 5 min to recover chloroplasts. The pellet was re-suspended in the same medium and centrifuged at 2000 × g for 10 min. Recovered chloroplasts were re-suspended and osmotically broken in 5 mM $MgCl_2$ followed by centrifugation at 2000 × g (10 min) in order to collect the thylakoid membranes (TMs). The prepared TMs were rinsed twice in a buffer containing 10 mM Tricine (pH 7.4), 5 mM $MgCl_2$, and 300 mM sucrose. The TMs were finally re-suspended in 10 mM sodium phosphate buffer (pH 7.4) containing 5 mM NaCl, 1 mM $MgCl_2$, and 100 mM sucrose to give a concentration of 2.9±0.2 mg chlorophyll per mL.[1]

8. Chlorophyll Content Determination

Chlorophyll content (C_{Ch}) was determined spectrophotometrically by re-suspending TMs in 100% acetone, as shown in Figure S3, and using the extinction coefficients and equation of Lichtenthaler.[2,3] The following equation (Eq. 1) was used for calculating the C_{Ch}:

$$C_{ch}(mg\ mL^{-1}) = \frac{8.02 \times A_{663} + 20.2 \times A_{645}}{10} \qquad \text{Eq. 1}$$

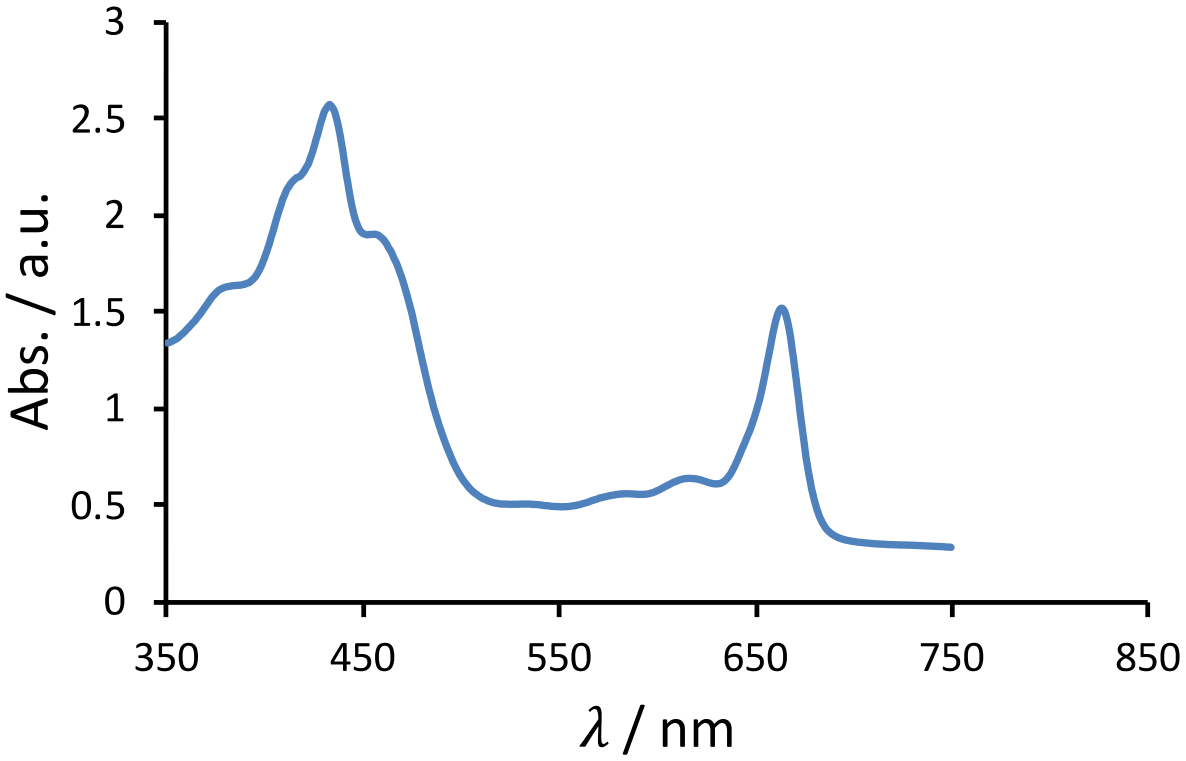

Figure S3. Absorbance spectrum of chlorophyll of thylakoid membranes (diluted 1:10 in acetone).

9. Composition of TMs

Samples for gel-electrophoresis analysis (BioRad, Hercules, CA, USA) were diluted (1:5) with a loading buffer. The SDS-PAGE running gel was prepared according to standard protocol reported in the literature.[4] The conditions of electrophoresis were 200 V for 55 min. Finally, the gel was stained in Coomassie brilliant blue R-250 as shown in Figure S4.[5] The SDS-PAGE pattern shown

in Figure S4 evidenced the main membrane protein complexes, i.e., those of Photosystem I and Photosystem II with their light harvesting complexes (LHC II), the oxygen evolution centers (OEC), the chlorophyll protein A (CPA), and the ATP synthase (ATPase).

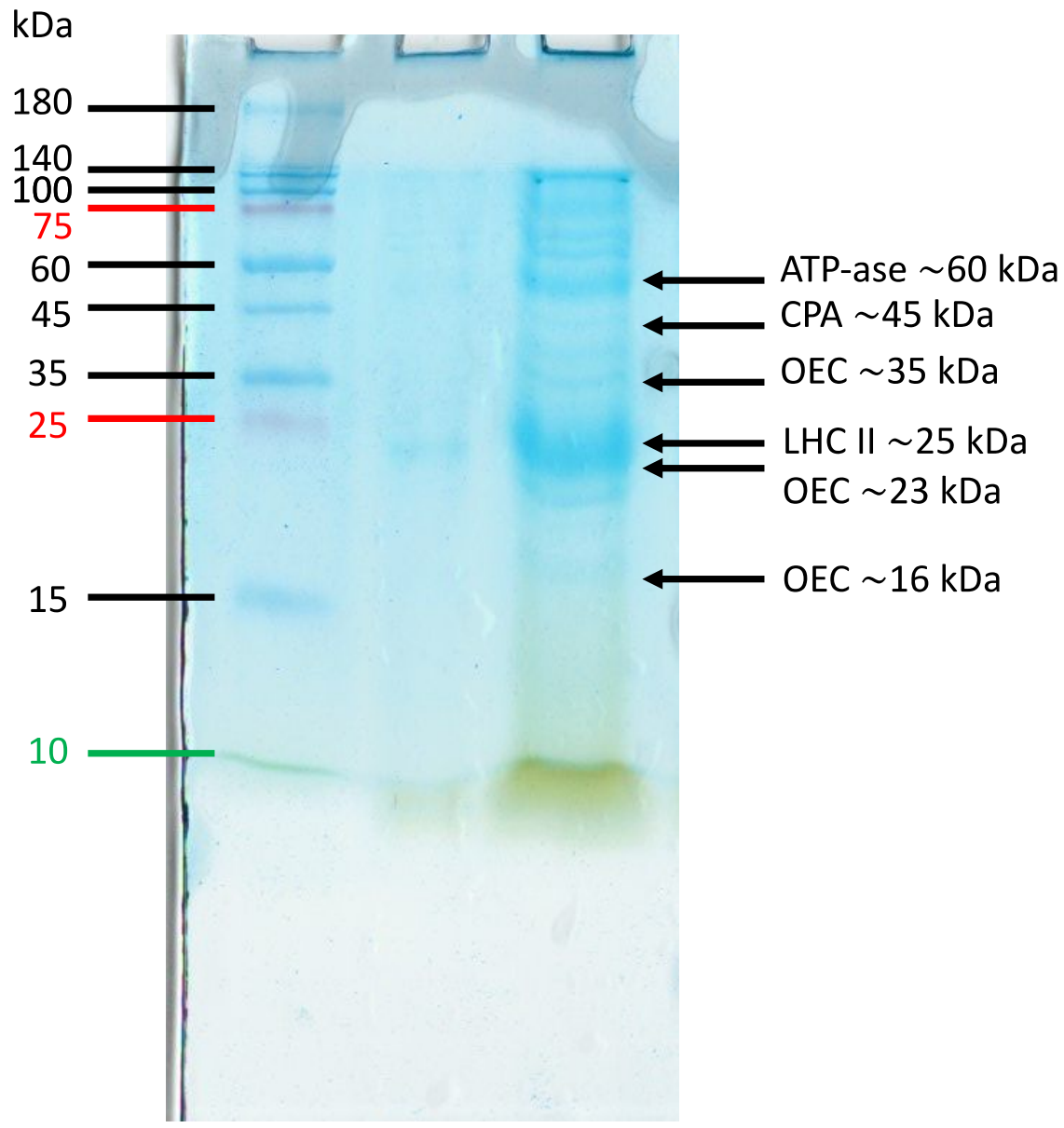

Figure S4. Gel-electrophoresis of the TMs.

References

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