Supporting Information

Electrochemical control of catalytic activity of immobilized enzymes

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Chemicals and materials

Enzymes: amyloglucosidase from *Aspergillus niger* (AMG; E.C. 3.2.1.3), glucose oxidase from *Aspergillus niger* (GOx; E.C. 1.1.3.4), horseradish peroxidase (HRP; E.C. 1.11.1.7), trypsin from porcine pancreas (E.C. 3.4.21.4), NAD^+-dependent glucose dehydrogenase from *Pseudomonas sp*. (GDH; E.C. 1.1.1.47); substrates: α-maltose, *N*α-benzoyl-DL-arginine 4-nitroanilide (BAPNA); β-nicotinamide adenine dinucleotide cofactor (oxidized form, NAD^+); reagents: 1-pyrenebutyric acid *N*-hydroxysuccinimidyl ester (PBSE), 2,2'-azino-*bis*(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (HEPES buffer), and other standard organic and inorganic materials and reagents were purchased from Acros Organics, Alfa-Aesar, Toyobo Enzyme, and MilliporeSigma (formerly Sigma-Aldrich). All commercial organic/inorganic chemicals, solvents and materials were used without further purification. 1-Pyrene butyrylpentafluorophenyl ester (PBPFE) was synthesized in-house by following the procedure described elsewhere.[1] All experiments were carried out in ultrapure water (18.2 MΩ·cm; Barnstead NANOpure Diamond) at room temperature (22 ± 2 °C). Buckypaper composed of compressed multiwalled carbon nanotubes (MWCNTs; Buckeye Composites, NanoTechLabs, Yadkinville, NC) was used as the electrode material.

Instrumentation

All UV-visible absorbance measurements were performed using VARIAN CARY Eclipse UV-visible spectrophotometer. Electrochemical measurements were carried out with an ECO Chemie

Autolab PASTAT 10 electrochemical analyzer, using the GPES 4.9 (General Purpose Electrochemical System) software package.

Immobilization of amyloglucosidase (AMG) on a buckypaper electrode:

A piece of buckypaper (0.5 cm × 0.2 cm) was rinsed with isopropanol for 15 minutes and then was immersed in 10 mM *N*-hydroxysuccinimidyl ester of pyrene-1-butyric acid (PBSE) solution in dimethyl sulfoxide (DMSO) for 1 hour. Then, the modified electrode with the adsorbed PBSE was rinsed with DMSO followed by 25 mM pH 6.0 HEPES buffer to remove the weakly bound part of PBSE. After that, the modified electrode was incubated in an AMG solution (2.3 mg/mL) in a HEPES buffer (25 mM, pH 6.0) for 2 hours under moderate shaking at room temperature (Figure SI1 shows the enzyme immobilization process schematically). The resulted modified electrode with a covalently bound AMG was rinsed with the HEPES buffer (25 mM, pH 6.0) for 30 minutes, changing the buffer every 10 minutes. The amount of the electrode-immobilized AMG was roughly estimated as ca. 0.2 U/cm^2 (vs. a geometrical electrode area) measuring the enzyme catalytic activity at pH 6.0.

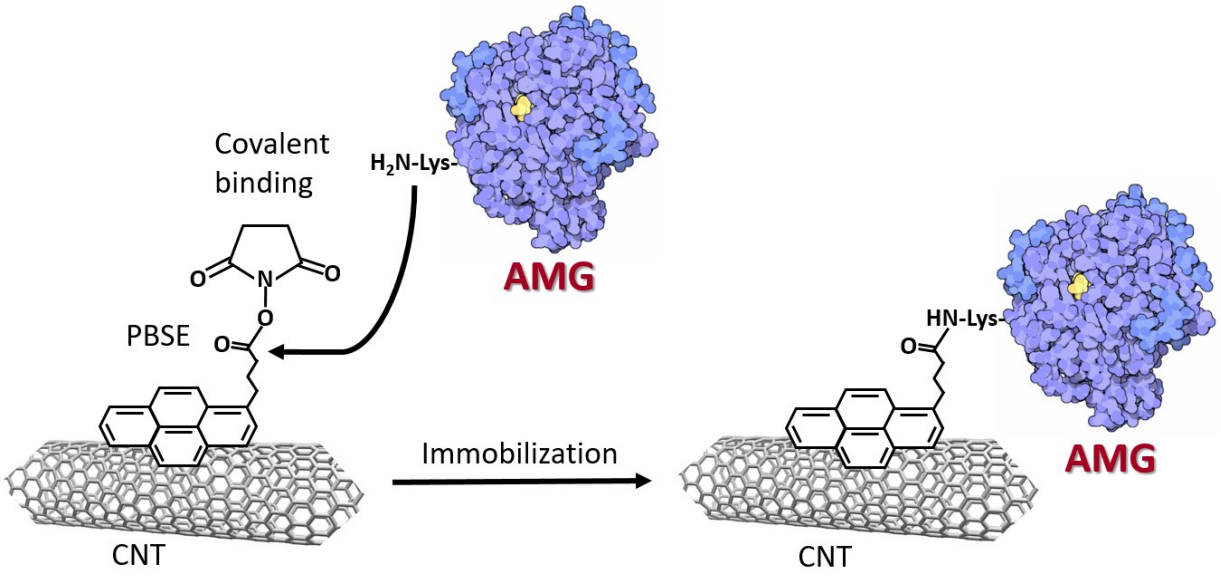

Figure SI1. The scheme of the AMG covalent immobilization on the buckypaper electrode (composed of carbon nanotubes, CNTs) using PBSE binding reagent.

Immobilization of trypsin on a buckypaper electrode:

A piece of buckypaper (0.5 cm × 0.2 cm) was rinsed with isopropanol for 15 minutes and then it was immersed in 10 mM 1-pyrenebutyryl pentafluorophenyl ester (PBPFE) solution in DMSO for 1 hour. The modified electrode was then rinsed with DMSO for 30 s, followed by rinsing with HEPES buffer (3 mM, pH 6.0) containing 10 mM calcium acetate and 0.1 M Na_2SO_4 to completely remove residual DMSO. The modified electrode was immersed in trypsin solution (10 mg/mL) in 25 mM HEPES buffer (pH 8.0) containing 10 mM calcium acetate and 0.1 M Na_2SO_4 for 1 hour under moderate shaking. Finally, the modified electrode with the covalently bound trypsin was rinsed with the HEPES buffer (3 mM, pH 6.0) containing 3 mM calcium acetate for 2 hours under moderate shaking, changing the buffer every 30 minutes (Figure SI2 shows the enzyme immobilization process schematically). The amount of the electrode-immobilized trypsin was roughly estimated as 0.01 U/cm^2 (vs. a geometrical electrode area) measruing the catalytic activity at pH 8.0.

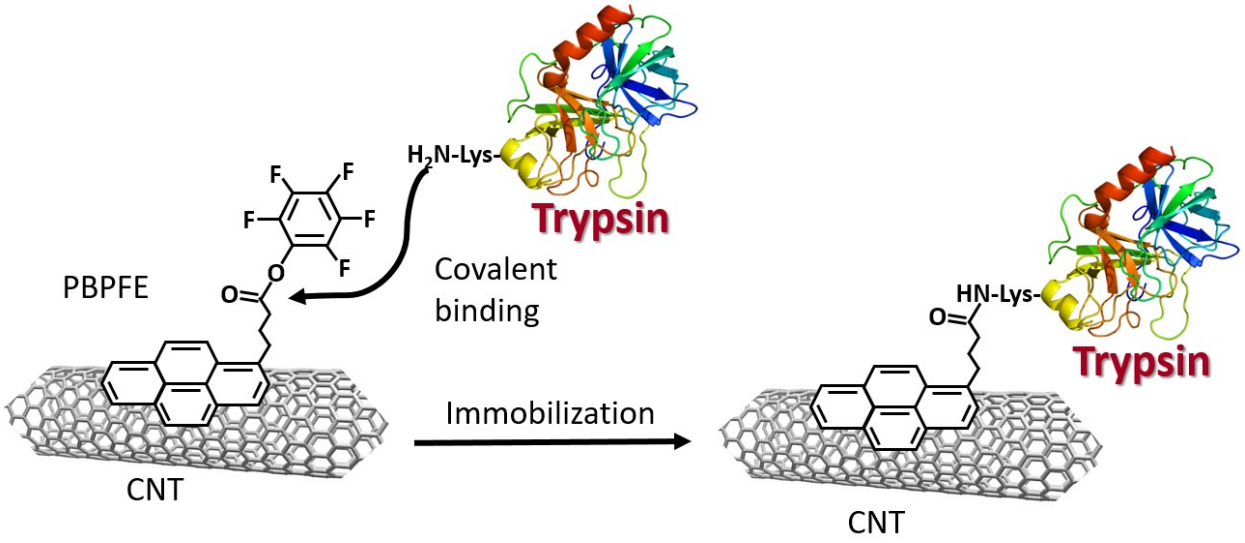

Figure SI2. The scheme of the trypsin covalent immobilization on the buckypaper electrode (composed of carbon nanotubes, CNTs) using PBPFE binding reagent.

It should be noted that the covalent coupling of enzymes/proteins through their reaction with surface-confined active esters may proceed at various pH values. The optimum pH for the amidation reaction depends on many factors and may vary from protein-to-protein. The

experimental conditions reported for the enzyme immobilization through the reaction similar to our procedure vary. For example, the used pH was 8.0,[2] 7.4,[3] or 7.0.[4] Specifically for the enzymes used in the present study the optimum pH in the course of the enzyme immobilization resulting in the best pH-controlled switching features was experimentally found as 6.0 for AMG and 8.0 for trypsin.

Amyloglucosidase (AMG) activity assay for the enzyme immobilized at a buckypaper electrode:

The activity of the electrode-immobilized AMG enzyme was analyzed in two steps:

(1) The AMG-modified buckypaper electrode was placed in an electrochemical cell containing α-maltose (2 mM) and 0.1 M Na_2SO_4 in a HEPES buffer (3 mM, pH 6.0 or 7.5 in the experiments specified below) and the enzyme-catalyzed reaction was allowed for 15 minutes. This experiment was performed under different conditions:

(a) in the presence of O_2 (in equilibrium with air) upon application of -0.5 V (vs. Ag|AgCl| KCl, 3M, reference electrode; note that all other potentials are reported vs. this reference) on the AMG-modified electrode; the HEPES buffer solution was set to the bulk pH 6.0;

(b) in the presence of O_2 (in equilibrium with air) upon disconnecting the AMG-modified electrode from a potentiostat (OCP – open circuit potential conditions); the HEPES buffer solution was set to the bulk pH 6.0;

c) the same as "a" and "b", but under anaerobic conditions obtained by bubbling Ar through the background solution; the HEPES buffer solution was set to the bulk pH 6.0;

d) in the presence of sodium ascorbate (1 mM) (also in the presence of O_2) upon application of +0.2 V on the AMG-modified electrode; the HEPES buffer solution was set to the bulk pH 7.5;

e) in the presence of sodium ascorbate (1 mM) (also in the presence of O_2) upon disconnecting the AMG-modified electrode from a potentiostat (OCP – open circuit potential conditions); the HEPES buffer solution was set to the bulk pH 7.5;

(2) After completing the first step, the solution containing different glucose concentrations was analysed for the presence of glucose. A solution aliquot (200 μL) was taken from the electrochemical cell and it was analyzed by two different methods depending on the solution composition:

(a) when the analyzed solution did not include ascorbate (the reaction was performed with or without oxygen), the glucose was assayed according to the standard procedure[5] using GOx-HRP-ABTS system; GOx (5 U/mL), HRP (5 U/mL) and ABTS (0.5 mM) in the HEPES buffer (100 mM, pH 7.4); the glucose concentration was calculated from the measured absorbance changes at λ = 420 nm corresponding to the production of the oxidized ABTS species (Figure SI3 shows schematically the reactions used in the assay process);

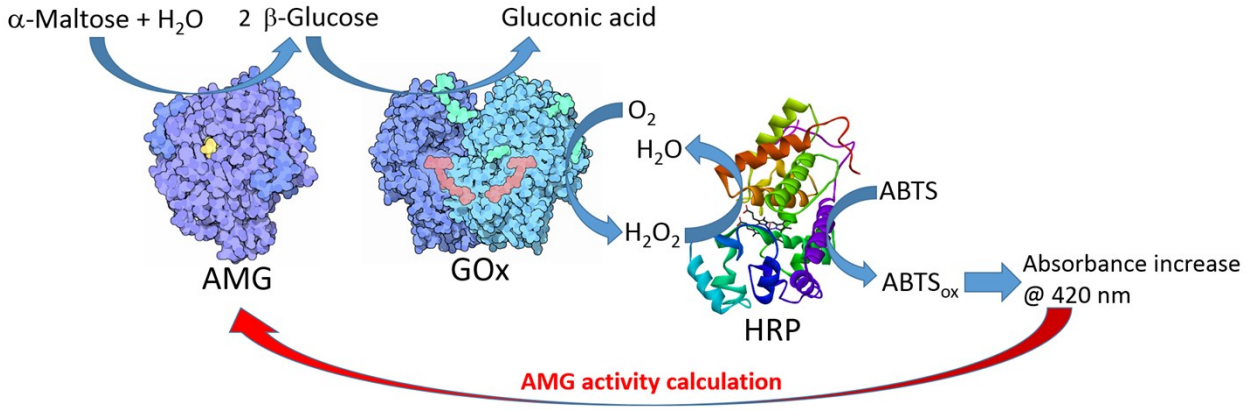

Figure SI3. The scheme of the AMG activity assay based on the $ABTS_{ox}$ production catalyzed by the GOx-HRP system.

(b) when the analyzed solution included ascorbate, the glucose was assayed[6] using NAD^+-dependent glucose dehydrogenase (GDH; 1 U/mL) and NAD^+ cofactor (2 mM) (note that the GOx-HRP-ABTS assay is not possible in the presence of ascorbate); the glucose concentration was calculated from the measured absorbance changes at λ = 340 nm corresponding to the production of the cofactor reduced form (NADH) (Figure SI4 shows schematically the reactions used in the assay process).

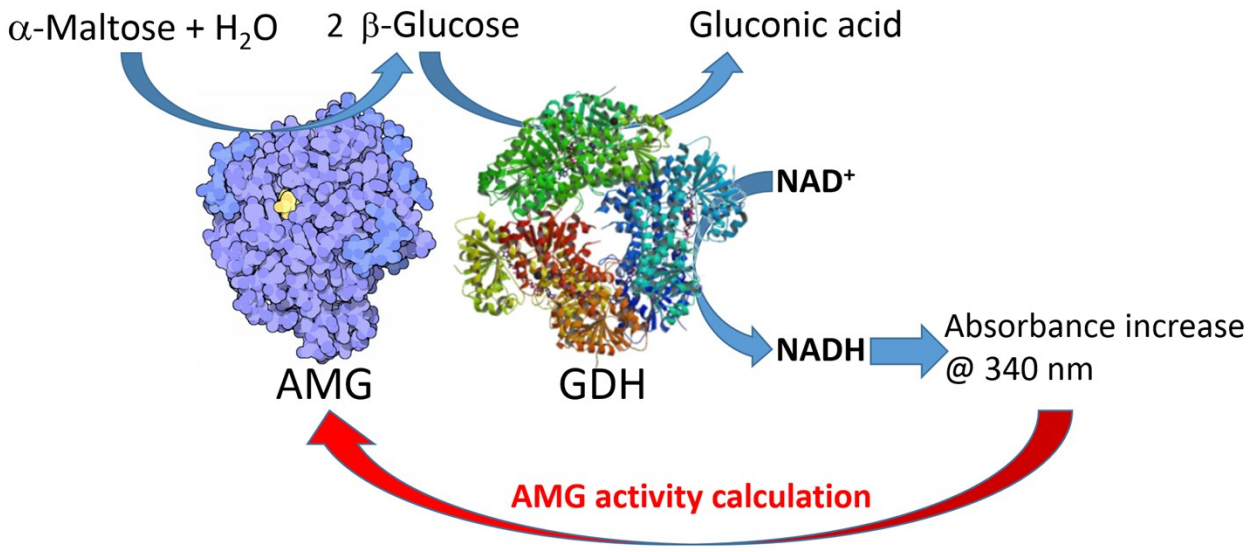

Figure SI4. The scheme of the AMG activity assay based on the NADH production catalyzed by GDH.

The electrochemically controlled (switchable) activity of the electrode-immobilized AMG:

The reversible activation–inhibition of the immobilized AMG was achieved by periodic application of -0.5 V potential on the modified electrode and its disconnection from the potentiostat (OCP conditions). The experiments were performed in the presence and absence of O_2. The exact composition of the reacting solution and the analysis of the enzyme activity are specified above.

Trypsin activity assay for the enzyme immobilized at a buckypaper electrode:

The activity of the electrode-immobilized trypsin enzyme was analyzed in two steps:

(1) The trypsin-modified buckypaper electrode was placed in an electrochemical cell containing *N*α-benzoyl-DL-arginine 4-nitroanilide (BAPNA, 1.14 mM) in Tris.HCl buffer (3 mM, pH 5.6) also containing 0.1 M Na_2SO_4 and 10 mM calcium acetate. The enzyme catalyzed reaction was allowed for 30 minutes. This experiment was performed under different conditions:

(a) in the presence of O_2 (in equilibrium with air) upon application of -0.5 V on the trypsin-modified electrode;

(b) in the presence of O_2 (in equilibrium with air) upon disconnecting the modified electrode from a potentiostat (the OCP condition);

(c) in the presence of ascorbate (1 mM) (also in the presence of O_2) upon application of +0.2 V on the trypsin-modified electrode.

(2) After completing the first reaction step, the solution aliquots containing different concentrations of the BAPNA hydrolyzed products was analyzed optically.[7] A solution aliquot (1 mL) was taken from the electrochemical cell, the optical absorbance change at λ = 405 nm corresponding to the production of 4-nitroaniline was measured, and the aliquot was returned to the cell. The rate of the absorbance changes was used to characterize the enzymatic activity of the immobilized trypsin (see Figure 5A in the paper).

The electrochemically controlled (switchable) activity of the electrode-immobilized trypsin:

The reversible inhibition-activation of the immobilized trypsin was achieved by periodic application of +0.2 V potential (for inhibition, when ascorbate was present in the background solution) on the modified electrode or its disconnection from the potentiostat (the OCP conditions for inhibition, when ascorbate was absent in the background solution) and application of -0.5 V potential (for activation). Note that these experiments were performed in the presence of O_2 (in equilibrium with air). The exact composition of the reacting solution and the analysis of the enzyme activity are specified above.

Effect of buffer concentration on the switchable function of the enzyme activity:

The buffer concentration is critically important to reveal the interfacial pH-controlled switchable features of the immobilized enzymes. Much lower buffer capacitance (significantly below the experimentally optimized buffer concentration of 3 mM) results in pH changes in the bulk solution following the primary interfacial pH change. In this case the local (interfacial) pH change cannot return back to the initial value when the potential applied on the electrode is released. On the other hand, the high buffer concentration (e.g., 100 mM) doesn't allow the interfacial pH changes, thus, inhibiting the enzyme activity variation. The optimum buffer concentration of 3 mM was found experimentally.

Confocal microscope images of a thin-film near the electrode surface with a variable local (interfacial) pH change

The fluorescence of 3,4'-dihydroxy-3',5'-bis-(dimethylaminomethyl)flavone (FAM345) is increasing with increasing of pH as reported in the literature.[8] In this experiment we observed a local pH increase at the electrode interface by applying -0.5 V at the working electrode in the presence of O_2 in the solution.The layer in which the local pH change occurred had a thickness of approximately 200 µm. The combination of electrochemical setup with fluorescent confocal microscope has already been used to investigate the thickness of the layer in which the pH change was occurring.[9] Note that in this preliminary study we used a graphite electrode instead of a buckypaper electrode.

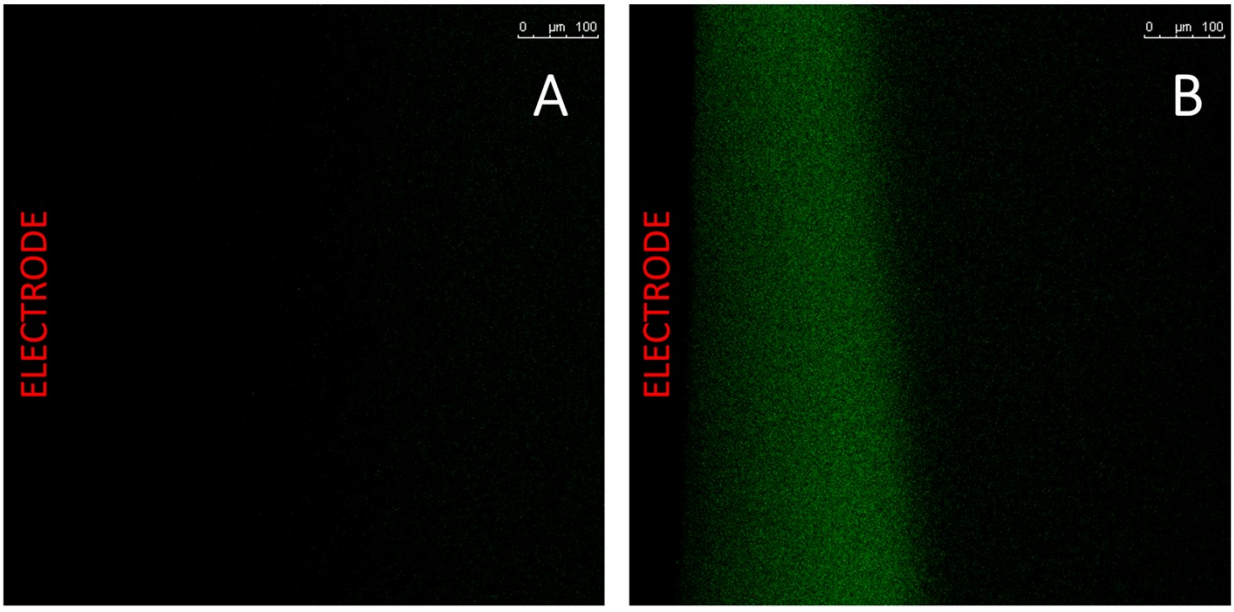

Figure SI5. Confocal microscope images of graphite electrode in 3 mM HEPES buffer with 100 mM Na_2SO_4 containing 1 µM 3,4'-dihydroxy-3',5'-bis-(dimethylaminomethyl)flavone: (A) before applying any potential and (B) after applying -0.5 V vs. Ag|AgCl (3M KCl) for 300 s. Experimental parameters: excitation wavelength λ_{ex}= 405 nm.

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