Self-Powered Biomolecular Keypad Lock Security System Based on a Biofuel Cell

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Experimental Section:

Chemicals and supplies.
Acid phosphatase (AP), type 1, from wheat germ (EC 3.1.3.2) was purchased from Sigma-Aldrich and purified as follows: 5 units of AP were soaked in 250 μL of 0.1 M sodium sulfate solution (pH 6.2) for 15 minutes, followed by centrifugation in a Nanosep 30 kDa ultra-filtration tube at 10000 rpm for 15 minutes at 22°C. The sediment was re-suspended in 150 μL of 0.1 M sodium sulfate solution (pH 6.2) and centrifuged again. The process was in total repeated three times. The centrifugation was performed with Microfuge® 22R Centrifuge from Beckham Coulter. All other enzymes were obtained from Sigma-Aldrich and used as supplied without any further purification: β-amylase (βAm) from sweet potato, type 1-B, (EC 3.2.1.2); maltose phosphorylase (MPh) from *Enterococcus* recombinant, expressed in *E. coli* (EC 2.4.1.8); glucose oxidase (GOx), type X-S, from *Aspergillus niger* (E.C. 1.1.3.4). Other chemicals purchased were of analytical quality and used as supplied from Sigma-Aldrich: β-D-(+)-glucose, 99.5% GC; poly(4-vinyl pyridine) (P4VP, M.W. 160 kDa) and starch (wheat purified). Bromomethyldimethylchlorosilane was purchased from Gelest. Sodium sulfate, anhydrous powder, 99%; potassium ferricyanide, anhydrous, 99.8%; and HPLC-grade toluene were from T.J. Baker. Hydrogen peroxide, 30% solution, reagent, ACS, was from VWR International, and ammonium hydroxide, ACS Plus, and sodium phosphate (dibasic anhydrous) were purchased from Fisher. Filtration of the working solution from enzymes was performed using a 6 mL centrifugal tube with a 10 kDa molecular weight cutoff (VIVASPIN 6 from Sartorius Stedim Biotech). Absolute ethanol was from Pharmco. Ultrapure water (18.2 MΩ cm) from NANOpure Diamond (Barnstead) source was used in all of the experiments. Single-side indium tin-oxide (ITO)-coated glass slides (20±5 Ω/sq surface resistivity) were purchased from Sigma-Aldrich.

Composition of the enzymatic keypad lock system and the input signals.
The enzymatic keypad lock system was composed of 8.3 mM starch, 10 mM potassium ferricyanide and 0.5 mM phosphate dissolved in 0.1 M sodium sulfate, pH 6.7, total reacting volume 6 mL. A starch solution was sonicated for 20 minutes prior to being used in the preparation of the initial reacting solution. The biocatalysts, βAm (619.2 U), MPh (33.4 U) coupled with AP (5 U) and GOx (423.6 U) were used as the input signals (**A**, **B** and **C**, respectively) to activate the concatenated **AND** logic gates. The reaction solution was allowed to incubate with the input **A**, βAm, for 2 hours before being transferred to a 6 mL centrifugal tube and centrifuged at 3500 rcf (relative centrifugal force) for one hour in order to separate the reacting solution from the enzyme-input **A**. The centrifugation was performed with IEC MULTI Centrifuge from Thermo Electron Corporation. The filtrate obtained after centrifugation was allowed to react with the next enzyme-input. The incubation and separation processes were

repeated for input **B** and **C** at 23±2°C. In order to increase the rate of starch hydrolysis, an elevated temperature, 50 ºC, was supplied to the reaction when βAm served as the enzyme-input signal. After the reaction solution was exposed to the three input signals, the final pH was measured followed by electrochemical and biofuel cell measurements.

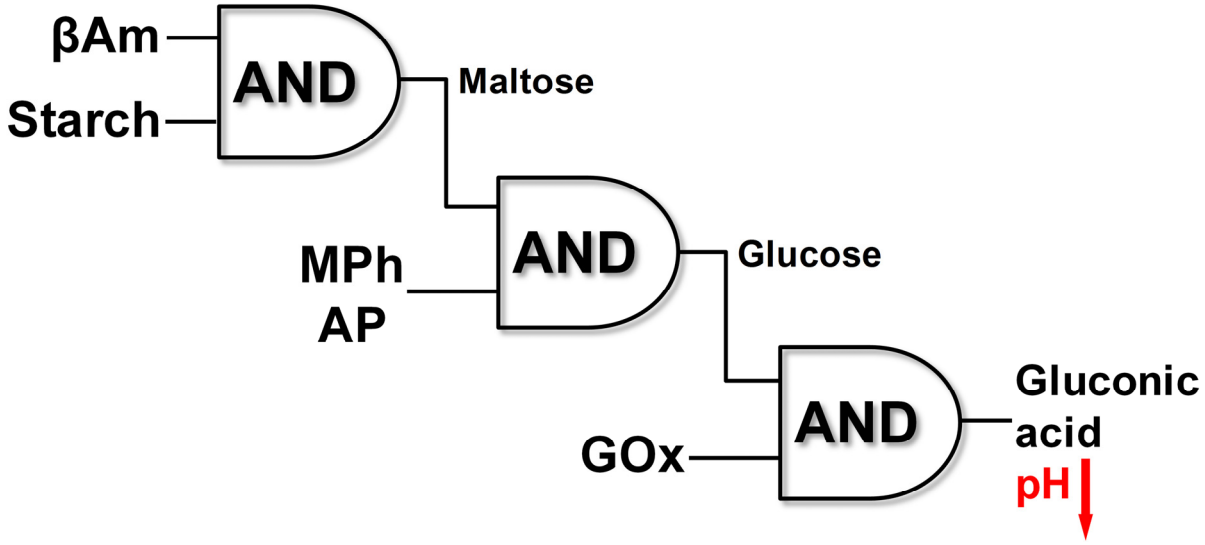
Electrode modification.
The commercially available indium-tin oxide (ITO) single-side coated glass slides were cut into 25 mm × 8 mm strips and then sonicated for 10-15 min in ethanol and dried under a stream of argon. The procedure was repeated with methylene chloride. The initial cleaning steps were followed by immersing the ITO glass for 1 hour into a cleaning solution (heated to 60 ºC in a water bath), composed of NH_4OH, H_2O_2, and H_2O in the ratio of 1:1:1 v/v (*Warning: This solution is highly reactive and extreme precautions must be taken upon its use.*). Subsequently, the glass strips were rinsed several times with water, immersed for 20 minutes in water at room temperature, and then dried under argon. The freshly cleaned ITO strips were then immersed for 20 minutes into a solution containing the silanization reagent, bromomethyldimethylchloro-silane, in toluene 0.1% (v/v) at 70 ºC. The silanized ITO was rinsed with several aliquots of toluene and dried under argon. Then 60 μL of the P4VP solution in nitromethane (10 mg mL^{-1}) were applied to the surface of each ITO glass strip, and left to react in a vacuum oven at 140 ºC overnight. The final cleaning steps, to remove the unbound polymer, consisted of soaking for 10 minutes in ethanol, followed by additional 10 minutes in a solution of diluted H_2SO_4 (pH 3.5). Modified electrodes were then stored under water.

The biofuel cell design.
The P4VP-modified ITO electrode (1.2 cm^2 geometrical area; note that the typical surface roughness factor for ITO electrodes is ca. 1.6±0.1 [1]),) was used as a pH switchable cathode using the solution composed of 0.1 M Na_2SO_4, 0.5 mM phosphate and 10 mM ferricyanide as an oxidizer after exposure to the enzymatic inputs. A bare ITO electrode (1.2 cm^2 geometrical area) applied as the anode for the glucose oxidation was used in 0.1 M phosphate buffer, pH 7.0, solution containing GOx (250 units mL^{-1}), methylene blue (0.1 mM) and glucose (100 mM) under Ar. The biofuel cell was custom made of two curved glass compartments (cathodic and anodic) forming a "U"-shaped configuration with clamping ledges separated by a Nafion® membrane (0.09 mm thick, Alfa Aesar, CAS # 66796-30-3). The voltage and current generated by the biofuel cell were measured by a multimeter (Meterman 37XR) using a variable resistance load. The measurements were carried out at ambient temperature (23±2 °C).

Instruments for the electrochemical and pH measurements.
Cyclic voltammetry measurements were carried out with an ECO Chemie Autolab PASTAT 10 electrochemical analyzer, using the GPES 4.9 (General Purpose Electrochemical System) software. Cyclic voltammograms were recorded in the potential range from +0.6 to 0 V. The potential scan rate was 100 mV s^{-1}. All the measurements were performed at room temperature (23 ± 2 °C), in a 5 mL electrochemical cell. The working electrode was the P4VP-modified ITO glass electrode with the geometrical area of 1.2 cm^2. A Metrohm Ag|AgCl|KCl, 3 M, electrode served as a reference electrode and a Metrohm Pt wire was used as a counter-electrode. The solutions (the filtrate) produced from the enzymatic keypad lock reactions, containing 10 mM ferricyanide as a redox probe, were used for the electrochemical measurements. The pH measurements were performed with Mettler Toledo SevenEasy pH-meter.

Ellipsometry measurements.
The layer thickness and the amount of the grafted material were evaluated at the wavelength of 633 nm and at the angle of incidence of 70° for the Si-wafers (modified similarly to the ITO-electrode and used for the ellipsometry measurements) using an Optrel Multiscop (Berlin, Germany) null-ellipsometer equipped with an XY-positioning table for mapping the sample surface (lateral resolution is defined by the beam spot of about 2 mm). From the ellipsometry measurements we calculated the grafting amount of the polymer, $A = H\rho$, and the grafting density, $\sigma = AN_A/M_w$, where H is the ellipsometric thickness of the dry brush sample, ρ is the density of the polymer (ρ = 1.101 g·cm^{-3} for P4VP), N_A is the Avogadro's number. The error in calculation for the ellipsometrically measured thickness is no larger than ±5% for the 5 nm thick films since the difference in the refractive indexes of all organic ingredients is small.

Equivalent logic circuitry.
The enzymes activating the reaction steps served as input signals for the system, which can be presented as the network composed of three concatenated **AND** gates. Each **AND** gate was activated by two input signals: one of them a simple chemical (starch, maltose and glucose) and the second is a biocatalyst (βAm, MPh coupled with AP, and GOx), Scheme SI-1.



Scheme SI-1. Representation of the enzyme-based keypad lock system as a network of three concatenated **AND** gates.

References:

[1] M.D. Carolus, S.L. Bernasek, J. Schwartz, J. *Langmuir* **2005,** *21,* 4236-4239.