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Enzyme Logic Gate Associated with a Single Responsive Microparticle: Scaling Biocomputing to Microsize Systems

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Supplementary Information

Chemicals and Materials. Chemicals purchased from Sigma-Aldrich (ACS quality) were glucose oxidase (GOx, from *Aspergillus niger*, type X-S, E.C. 1.1.3.4), esterase (Est, from porcine liver, E.C. 3.1.1.1), urease (from jack beans, E.C. 3.5.1.5), high-molecular weight poly(vinyl chloride) (PVC; M.W. ca. 200 kDa), *bis*(2-ethylhexyl) sebacate (DOS), *tert*-butyl calix[4]arene tetraethyl ester (sodium ionophore X), sodium *tetrakis*[3,5-*bis*(trifluoromethyl)phenyl borate (NaTFPB), 9-(diethylamino)-5-octadecanoylimino-5H-benzo[a]phenoxazine (chromoionophore I, ETH 5294), tetrahydrofuran (THF), cyclohexanone, ethylene glycol diethyl ether, polyoxyethylene (23) lauryl ether (Brij-35), acetone, 3-(trimethoxysilyl)propyl methacrylate (Silane A174), D-(+)-glucose, ethyl butyrate and urea.. All other chemicals were purchased from VWR (West Chester, PA). All aqueous solutions were prepared with ultrapure water (18.2 MΩ·cm) from Milli-Q water purification system (Millipore).

Preparation of Membrane Cocktail. The optode microparticle components, the polymer (PVC) and the plasticizer (DOS) were dissolved in 1 mL of a 2:1 mixture of cyclohexanone and ethylene glycol diethyl ether. The cocktail contained 40 $mmol\cdot kg^{-1}$ sodium ionophore X, 20 $mmol\cdot kg^{-1}$ ion-exchanger NaTFPB, and 10 $mmol\cdot kg^{-1}$ chromoionophore I. THF was added to dilute the polymer solution in the ratio of 1:2.

Fabrication of the Optode Microparticles. A solvent displacement method described recently was used to produce the optodes. [1] A small (~ 8 mL) vial was filled with an aqueous solution of a surfactant (0.01% wt. Brij-35). The solution was stirred using a small magnetic stir bar at ca. 100 rpm. The membrane cocktail solution was drawn into a disposable syringe with the attached needle. The syringe was held above the vial, the needle was not immersed into the aqueous phase being positioned at 4-5 mm above the solution surface, and the membrane cocktail solution (100 μL) was rapidly injected. Instantaneous precipitation produced a stable milky emulsion often with a small fraction of a coagulum. The emulsion was filtered using a Whatman No. 1 filter, and then diluted 10-fold with water to yield the optode microparticles emulsion.

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Optode Microparticles Immobilization. Square cover glass strips (22 × 22 mm) were sonicated in 0.1 M NaOH solution for 10 min, rinsed with water and then sonicated in water for 2 min. The glasses were then rinsed with acetone and kept in an oven at 120 ˚C for 15 min. The cover glass holder was put into a desiccator with Silane A174 and kept under vacuum for 1 h. A small droplet of the optode microparticles emulsion was deposited on the "wet" cover glass and allowed to dry completely. The cover glasses were conditioned in a 0.1 M TRIS solution at pH 7.5 for 24 hours.

Instrumentation and Measurements. The custom-made flow cell (similar to VacuCel™ produced by C&L Instruments, Hershey, PA) was used to hold a microscope cover glass. The cell was connected to a peristaltic pump (Variable-Flow MiniPump, Fisher Scientific, Pittsburgh, PA) operating at the rate of 0.1 $mL \cdot s^{-1}$. The measurements were carried out in a continuous flow mode. The pH was measured using pH-meter (Accumet XL-15, Fisher Scientific, Pittsburgh, PA) with a double-junction combination glass pH-electrode. The optical setup included an inverted fluorescence microscope (Olympus IX-71, Olympus, Center Valley, PA) with attached microspectrometer (Acton Microspec MS-2150) and PIXIS-512 cooled CCD camera (both from Princeton Instruments, Trenton, NJ). The microspectrometer allowed simple switching between direct imaging (non-dispersed) and spectral imaging (dispersed) modes. A mirror and a diffraction grating were attached to the motorized computer-controlled turret providing fast switching capability; thus, the same camera was used for both direct and spectral imaging. A fast wavelength switch DG-4 (Sutter Instrument, Novato, CA) with a 300 W xenon arc lamp and a 535 (±25) nm filter was used as a light source. A filter cube consisted of a 565 nm dichroic mirror and a 600 nm long-pass emission filter. The microscope was equipped with 40x/0.17 objective (UPlanSApo, Olympus, Center Valley, PA). The camera and the spectrometer were controlled by a PC running WinSpec32 software (Princeton Instruments, Trenton, NJ) in a slave mode. A custom-programmed microcontroller was used to control DG-4 and generate triggering signals for the CCD camera. The detection was performed with a 220 ms pulse of excitation light (550 nm) with simultaneous triggering of the camera shutter for 200 ms exposure. At first, the microspectrometer was switched in the direct imaging mode. To avoid photobleaching, continuously acquired image was examined in the transmission mode and a microparticle of interest was selected. Then the microscope stage was moved in order to position the microparticle in the center, and the slit was placed at the spectrometer entrance. The setup was switched to the fluorescence mode, and the fluorescence spectrum was acquired. The microscope table was controlled by a home-built combination of the motorized stage (H107, Prior Scientific, Rockland, MA) and piezoelectric stage (P517-K024, Physik Instrumente, Irvine, CA). The mechanical positioner allowed a coarse displacement within ± 20 mm with ± 1 µm resolution. The piezoelectric stage had a 100 µm throw with a 1 nm closed-loop resolution. In practice the latter

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was limited to ±25 nm due to the limited resolution of the 12-bit DAC (USB-6009, National Instruments, Austin, TX) used to control analog piezoelectric stage controller. The long-pass filter in the fluorescence cube allowed us to record the spectra within the 600-800 nm region. The fluorescence of the chromoionophore I in the optode microparticle was measured at 650 and 680 nm that correspond to the fluorescence maxima of the deprotonated and protonated forms of the dye. For calibrating the optode microparticle, aqueous solutions containing 0.1 M Na_2SO_4 and a universal buffer (3.3 mM citric acid, 5.5 mM boric acid and 5.0 mM phosphoric acid) were titrated to various pH values with 1 M KOH solution. All experiments were conducted at ambient temperature (23±2 °C). Activity coefficients were calculated according to Debye-Hückel formalism. [2]

Table 1 summarizes the pH values: before and after different input combinations. Note that the pH values generated in situ by the enzyme system were measured by individual optode microparticles.

Input combination	**0,0**	**0,1**	**1,0**	**1,1**
Microparticle size, μm	6.0	7.0	7.0	6.5
Initial pH	6.51	6.39	6.27	6.33
Final pH	6.44	5.17	4.00	4.30
ΔpH	0.07	1.22	2.27	2.03

References:

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