A model system for targeted drug release triggered by biomolecular signals logically processed through enzyme logic networks

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Electronic supplementary information (ESI)

Chemicals and reagents used:
Maltose phosphorylase (MPh; E.C. 2.4.1.8) from *Enterococcus sp*., hexokinase (HK; E.C. 2.7.1.1) from *Saccharomyces cerevisiae*, glucose-6-phosphate dehydrogenase (G6PDH; E.C. 1.1.1.49) from *Leuconostoc mesenteroides*, glucose dehydrogenase (GDH; E.C. 1.1.1.47) from *Pseudomonas sp.*, alcohol dehydrogenase (AlDH; E.C. 1.1.1.1) from *Saccharomyces cerevisiae*, sodium alginate from brown algae (medium viscosity, ≥2000 cP), pyrroloquinoline quinone (methoxatin disodium salt, PQQ), albumin (from bovine serum, BSA), β-nicotinamide adenine dinucleotide sodium salt (NAD^+), β-nicotinamide adenine dinucleotide reduced dipotassium salt (NADH), poly(ethyleneimine) solution (PEI), D-(+)-maltose monohydrate, adenosine 5'-triphosphate disodium salt hydrate (ATP), D-(+)-glucose, glucose-6-phosphate, 3,3',5,5'-tetramethylbenzidine dihydrochloride (TMB), (1-ethyl-3[3-(dimethylamino)propyl] carbodiimide (EDC), *N*-hydroxysuccimide (NHS), (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES-buffer), 2-amino-2-hydroxymethyl-propane-1,3-diol (Tris-buffer) and other standard organic and inorganic materials and reactants were obtained from Sigma-Aldrich or J.T. Baker and used without further purification. AffiniPure horseradish peroxidase-labeled anti-goat IgG-antibody from donkey (HRP-Ab) and AffiniPure anti-rabbit IgG-antibody from goat (used as AG) were purchased from Jackson ImmunoResearch. All experiments were carried out in ultrapure water (18.2 MΩ·cm; Barnstead NANOpure Diamond).

Instrumentation:
A Shimadzu UV-2450 UV–Vis spectrophotometer with 1 mL poly(methyl methacrylate) (PMMA) cuvettes was used for optical spectrum measurements and a BIO RAD Model 680 ELISA microplate reader was used for all optical measurements of the HRP-immuno-assay. Electrochemical experiments (cyclic voltammetry and application of controlled potential on the alginate-electrode) were performed in a 3-electrode cell: Metrohm Ag|AgCl|KCl, 3M, reference, graphite counter, and graphite working electrode. A single compartment cell was used with an electrochemical workstation (ECO Chemie Autolab PASTAT 10) and GPES 4.9 (General Purpose Electrochemical System) software.

Experimental procedures:
Deposition of alginate on a graphite support. Sodium alginate (1.5% w/w) was dissolved in 100 mM Na_2SO_4 (pH 6.0) and stirred for 30 minutes at 45 °C. The solution was cooled to room temperature and $FeSO_4$ (35 mM) and HRP-Ab (16 μg mL^{-1}) were added and mixed well. The HRP-Ab was entrapped into the Fe^{+3}-alginate film upon its electrochemical deposition on the electrode surface. The mixture was deposited on a graphite electrode upon oxidation by a potentiostat (+0.8 V, 60 sec) of Fe^{2+} cations,

yielding Fe^{3+} and resulting in an alginate cross-linked film on a graphite surface containing entrapped HRP-Ab. The resulting electrode was washed thoroughly with water. The detailed thickness analysis and electrochemical characterization were reported elsewhere.[1]

Functionalization of graphite with PQQ. Graphite electrodes (pencil rods)[2] were polished with sandpaper until appearing smooth, rinsed, and incubated in 1% w/w PEI solution for 20 mins. Following, the electrodes were washed 3 times in water and incubated in a solution containing EDC (50 mM), NHS (50 mM), and PQQ (20 μM) made in HEPES-buffer (10 mM, pH 7.5) for 3 hours. The electrodes were then washed and stored in HEPES-buffer (10 mM, pH 7.5). The details of the PQQ immobilization procedure and the modified electrode characterization were reported elsewhere.[3]

Potential measurements of the PQQ electrode vs. an Ag|AgCl|KCl 3M (Metrohm) reference electrode were performed in Tris-buffer (100 mM, pH 7.4, containing 20 mM $CaCl_2$; note the use of Ca^{2+} cations for promoting NADH oxidation by PQQ[4]) in the absence of NADH and generation of NADH *in situ* through biocatalytic reactions. The potential measurements were performed under steady-state conditions in the open circuit vs. the reference electrode.

Immuno-assay of released HRP-Ab. An ELISA plate was incubated overnight at 4 °C containing 100 μL per well of AG (10 μg/μL) made in carbonate buffer (50 mM, pH 9.5). The plate was then washed 3 times with 0.1 M phosphate buffered saline (PBS), pH 7.4, containing 0.05% Tween. The plate was then blocked using 200 μL of 0.1% BSA solution per well (made in 0.1 M PBS, pH 7.4) incubated for 1 hr. The washing step was repeated and the solution containing HRP-Ab released from the alginate cross-linked matrix was added to each well and incubated for 1 hr. The washing step was completed and the HRP-Ab bound to AG was detected using an optical assay. A solution composed of 50 μL H_2O_2 (0.035 % v/v), 30 μL TMB (4 mM in DMSO) and citrate buffer (100 mM, pH 5.5) was prepared for the analysis of the released HRP-Ab bound AG complex. This solution was added to the ELISA plate wells and an increase in absorbance at λ = 655 nm was measured using a microplate reader (BIO RAD 680). In the control experiments the released HRP-Ab was added to the ELISA well without deposited AG.

Operation of 4-input-AND concatenated gates. Maltose (Input A, 2 mM for logic value **1**), sodium phosphate (Input B, 2 mM for logic value **1**), ATP (Input C, 1 mM for logic value **1**), NAD^+ (Input D, 2 mM for logic value **1**), MPh (2 U/mL), HK (2 U/mL), and G6PDH (2 U/mL) were combined in Tris-HCl buffer (100 mM, pH 7.4, containing 20 mM $CaCl_2$) and used to generate NADH *in situ*. Logic values **0** for the inputs A-D were defined as the absence of the corresponding chemicals. This solution was allowed to incubate for 30 mins at 37 °C and then used to release HRP-Ab from an alginate matrix using the PQQ-modified electrode.

Operation of 3-input-OR gate. Glucose (Input A, 2 mM for logic value **1**), ethanol (Input B, 85 mM for logic value **1**), glucose-6-phosphate (Input C, 1 mM for logic value **1**), NAD^+ (1.5 mM), GDH (1 U/mL), AlDH (4 U/mL), and G6PDH (50 mU/mL) were combined in Tris-HCl buffer (100 mM, pH 7.4, containing 20 mM $CaCl_2$) and used to generate NADH *in situ*. Logic values **0** for the inputs A-C were defined as the absence of the corresponding chemicals. The solution was allowed to incubate for 30 mins at 37 °C and then used to release HRP-Ab from an alginate matrix using the PQQ-modified electrode.

References:

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