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Supplemental material for

Investigating DNA hydrogels as a new biomaterial for enzyme immobilization in biobatteries

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Materials and methods. All reagents were obtained from commercial sources and used without further purification unless otherwise stated. Glucose oxidase from Aspergillus niger (EC 1.1.3.4, type X-S, 157 U/mg of solid, 75% protein) was purchased from Sigma. DNA strands were synthesized and purified at the University of Utah Core Facility. The DNA sequences used in this work are provided in Table S1.

*DNA-enzyme Bioanode preparation***.** Toray carbon paper electrodes were cut into L-shapes and coated with paraffin wax at the connecting ends to have 0.25 cm^2 exposed electrode area. The film casting solution contain 100 μM Y-DNA, 125 μM L-DNA, 10 mg/ mL glucose oxidase in PBS buffer (pH 7). Then, 7 μL of the film solution was drop cast onto the exposed area of Toray electrodes. Electrodes were dried at room temperature for 2 h.

*Electrochemical studies***.** Electrodes constructed as described above were analyzed using a conventional three-electrodes set up with Ag/AgCl electrode and platinum mesh as the reference and counter electrodes, respectively. Electrochemical studies were carried out with the CH650 potentiostat. Electrodes were immersed in to PBS buffer (pH 7) for 10 minutes to remove non-adsorbed enzyme on the hydrogel surface and equilibrated in PBS buffer (pH 7) containing 0.1 mM ferrocenecarboxylic acid for 5 minutes before each measurement. The bioelectrocatalytic activity of the DNA-enzyme hydrogel was investigated by cyclic voltammetry (from 0.1 V to 0.5 V *vs.* Ag/AgCl at 10 mV/sec) in the absence or presence of 20 mM glucose.

Enzymatic biobattery construction. The DNA-enzyme hydrogel was prepared by combining Y-DNA (1mM), L-DNA (1.25 mM), glucose oxidase (30 mg/mL), Fc-COOH (0.5 mM) and Na_2SO_4 (1 M) in a total volume of 4 μL. The enzymatic biobattery was constructed as previously described. Briefly, the biohydrogel was first spread onto 0.125 cm^2 stainless-steel mess surface to make an anode. The cathode consists of an ELAT gas diffusion electrode containing 20% Pt on Vulcan XC-72 (E-Tek) hot pressed to Nafion NRE212 membrane. The cathode was then assembled with the anode and both electrodes are exposed to air. The biobattery was tested by adding 1 μL of 500 mM glucose, measuring the open circuit potential (OCV) and recording the polarization curve by linear sweep voltammetry from OCV to 0.001V at 50 mV/sec.

Table S1. DNA sequences studied. Y1, Y2, Y3 are used to construct Y-DNA. L1, L2 are used to construct L-DNA.[1]

Name	Sequences
Y1	5'-CGATTGACTCTCCACGCTGTCCTAACCATGACCGTCGAAG-3'
Y2	5'-CGATTGACTCTCCTTCGACGGTCATGTACTAGATCAGAGG-3'
Y3	5'-CGATTGACTCTCCCTCTGATCTAGTAGTTAGGACAGCGTG-3'
L1	5'-GAGAGTCAATCGTCTATTCGCATGAGAATTCCATTCACCGTAAG-3'
L2	5'-GAGAGTCAATCGCTTACGGTGAATGGAATTCTCATGCGAATAGA-3'

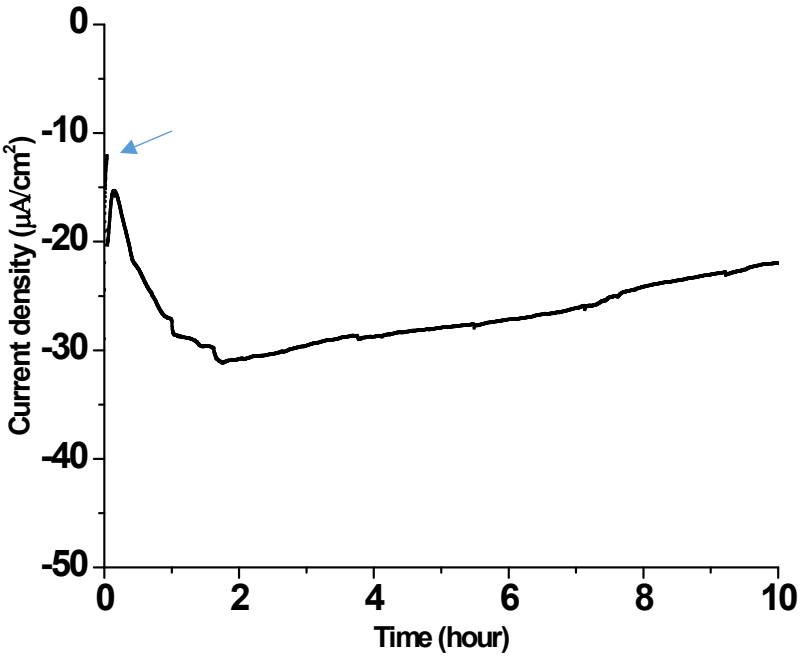

Figure S1. Stability of the bioanode as a function of the current density over times. The arrow indicates the addition of 20 mM glucose and the bioanode was poised at 0.36 V *vs.* Ag/AgCl.

References

1. Y. Xing, E. Cheng, Y. Yang, P. Chen, T. Zhang, Y. Sun, Z. Yang and D. Liu, *Advanced Materials*, 2011, **23**, 1117-1121.