A Nanofabricated Enzyme Biosensor

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The demonstration of a nanofabricated biofuel cell using individual carbon nanotube electrodes functionalized with enzymes offers a unique platform to study catalysis at the single enzyme level.[1] Of key importance is how the nanofabrication leads to a device geometry that constrains the location and function of the enzymes. This is shown dramatically in Figure 1 where the schematic of the reported biofuel cell is contrasted with the device geometry drawn to scale. The first constraint is in that the nanoscale window leading to the metal contact limits the electrophoretic deposition to only a single carbon nanotube.[2] Length fractionation of the carbon nanotubes also constrains the end of the carbon nanotube as the only location of bonding sites for the enzyme to attach. Cyclic voltammetry is used to deposit the enzymes on specific carbon nanotubes. Since the enzymes are polarized, the electric field used in their deposition will orient them to favor certain available amine groups that can bind with carboxyl groups at the end of the carbon nanotube. The enzymes are relatively large molecules compared to the 1 nm diameter nanotube. Therefore, once an enzyme attaches, there is no space on the end of the nanotube to attach another enzyme. That is, the combination of nanofabrication and directed self-assembly leads to a single carbon nanotube with an electrical connection to a single enzyme.

The architecture in Figure 1 may be considered as a two-enzyme sensor for glucose that outputs a current which is a function of glucose concentration. It differs from an amperometric sensor in that no external potential is applied to measure the current. We have performed a series of measurements and calculations with this system. Some of the results are surprising. One in particular is that when the glucose/oxygen reaction starts there is an initial peak in the current followed by an exponential-like decay over ~ 3 minutes into a 200 TOhm load. This decay can be interpreted as caused by a decrease in local reactants (i.e. fuel) as the reaction becomes diffusion limited. However, the same peak response with decay is evident when fluid with reactants are continuously flowed over the active region of the device. This behavior is repeatable with several devices that have been tested over a period of up to 90 days. The behavior derives from a combination of the unique architecture of the device, the fundamentals of the reaction kinetics, and the laminar flow condition. The current measured between the two enzymes is consistent with a theoretical model for this architecture.

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[1] A. Kanwal, S. C. Wang, Y. Ying, R. Cohen, S. Lakshmanan, A. Patlolla, Z. Iqbal, G. A. Thomas and R. C. Farrow, Electrochemistry Communications **39**, 37-40 (2014).

[2] A. Goyal, S. Liu, Z. Iqbal, L. A. Fetter and R. C. Farrow, J. Vac. Sci. Technol. B **26**, 2524-2528 (2008).

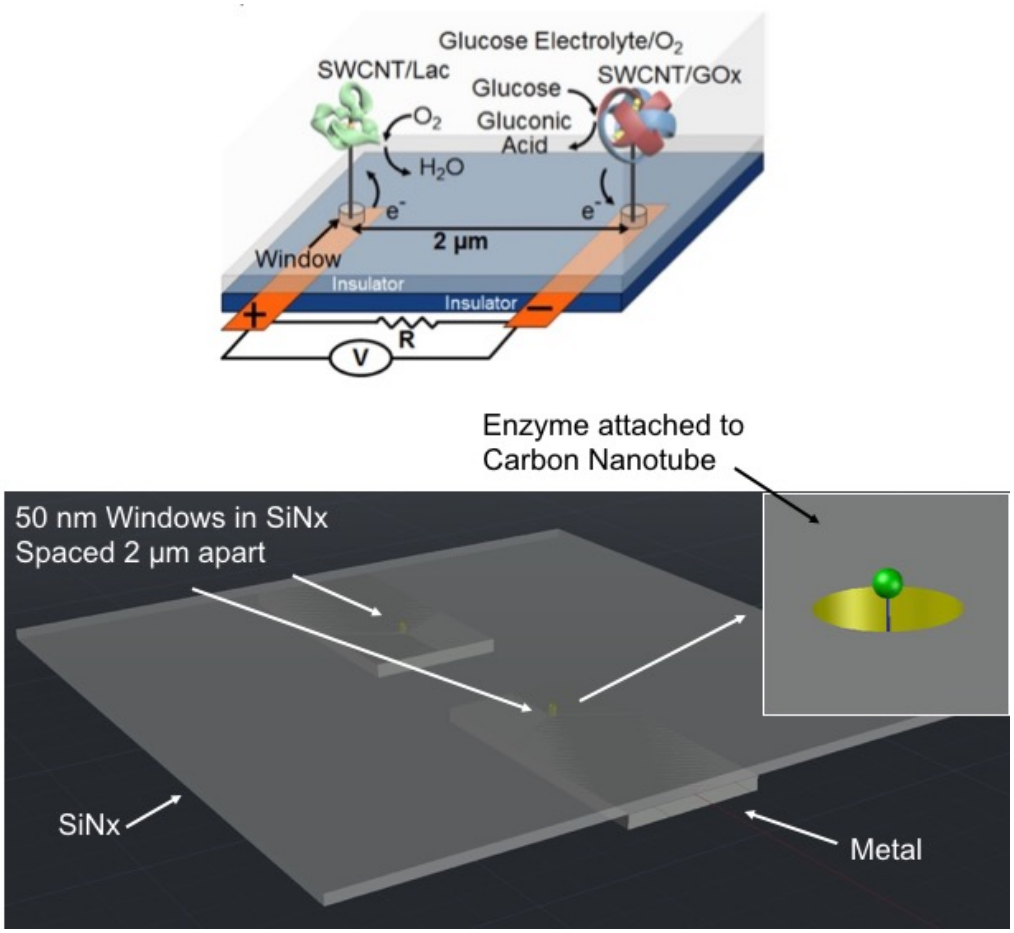

Figure 1: Schematic diagram of biofuel cell concept (top) and layout drawn to scale (bottom). The layout is shown without topography of SiNx over metal tracks. The inset shows the position of a deposited enzyme assuming that the window depth is 75 nm and that an average length carbon nanotube (83 ± 26 nm) is deposited.

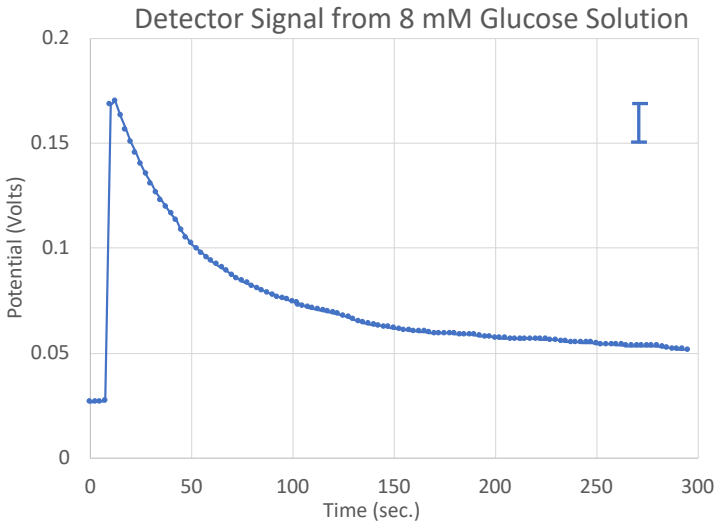

Figure 2: Potential measured between glucose oxidase and laccase enzymes in a solution of 8 mM glucose in phosphate buffer (pH 7) flowing at 200 µL/min (average of 4 measurements from a single device).