# Electrochemical Glucose Biosensor of Platinum Nanospheres Connected by Carbon Nanotubes

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## Abstract

### Background:

Glucose biosensors comprised of nanomaterials such as carbon nanotubes (CNTs) and metallic nanoparticles offer enhanced electrochemical performance that produces highly sensitive glucose sensing. This article presents a facile biosensor fabrication and biofunctionalization procedure that utilizes CNTs electrochemically decorated with platinum (Pt) nanospheres to sense glucose amperometrically with high sensitivity.

### Method:

Carbon nanotubes are grown *in situ* by microwave plasma chemical vapor deposition (MPCVD) and electrochemically decorated with Pt nanospheres to form a CNT/Pt nanosphere composite biosensor. Carbon nanotube electrodes are immobilized with fluorescently labeled bovine serum albumin (BSA) and analyzed with fluorescence microscopy to demonstrate their biocompatibility. The enzyme glucose oxidase ($GO_X$) is immobilized onto the CNT/Pt nanosphere biosensor by a simple drop-coat method for amperometric glucose sensing.

### Results:

Fluorescence microscopy demonstrates the biofunctionalization capability of the sensor by portraying adsorption of fluorescently labeled BSA unto MPCVD-grown CNT electrodes. The subsequent $GO_X$–CNT/Pt nanosphere biosensor demonstrates a high sensitivity toward $H_2O_2$ (7.4 µA/mM/cm$^2$) and glucose (70 µA/mM/cm$^2$), with a glucose detection limit and response time of 380 nM (signal-to-noise ratio = 3) and 8 s ($t_{90\%}$), respectively. The apparent Michaelis–Menten constant (0.64 mM) of the biosensor also reflects the improved sensitivity of the immobilized $GO_X$/nanomaterial complexes.

### Conclusions:

The $GO_X$–CNT/Pt nanosphere biosensor outperforms similar CNT, metallic nanoparticle, and more conventional carbon-based biosensors in terms of glucose sensitivity and detection limit. The biosensor fabrication and biofunctionalization scheme can easily be scaled and adapted for microsensors for physiological research applications that require highly sensitive glucose sensing.

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**Abbreviations:** (BSA) bovine serum albumin, (CNT) carbon nanotube, (FITC) fluourescein isothiocyanate, ($GO_X$) glucose oxidase, ($H_2O_2$) hydrogen peroxide, (MPCVD) microwave plasma chemical vapor deposition, (NADH) nicotinamide adenine dinucleotide, (PAA) porous anodic alumina, (PBS) phosphate buffered saline, (Pt) platinum

**Keywords:** carbon nanotubes, fluorescence, glucose biosensor, platinum nanospheres

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## Introduction

Diabetes mellitus is a metabolic disease marked by high levels of blood glucose that can lead to serious complications, including kidney failure, blindness, cardiovascular disease, and premature death.[1] An active glucose monitoring and control regime is required of all diabetes patients to maintain a healthy lifestyle and prevent diabetes-related complications. Furthermore, tightly controlling glucose levels in critically ill patients with diabetes and even without diabetes has shown to reduce numerous medical complications and premature death.[2,3] A highly sensitive glucose biosensor could improve the sensitivity and accuracy of current blood glucose monitoring technologies since reports by the Food and Drug Administration suggest raising standards in home glucose monitors, including glucose testing strips and meters, to improve their glucose detection accuracy.[4] Therefore, a glucose monitoring technology with increased glucose sensitivity and accuracy could substantially improve the prognosis of diabetes and critically ill patients and subsequently reduce their associated health care expenses. Thus the focus of glucose monitoring technologies has been on treating diabetes instead of elucidating the fundamental mechanisms behind abnormal insulin secretion in pancreatic islets and β cells.

Insulin production within pancreatic islets and β cells is dependent on proper glucose uptake and metabolism,[5–7] and therefore, highly sensitive glucose biosensors that monitor cellular glucose transport processes would be of great benefit to fundamental diabetes research. The signaling pathway controlling insulin release is a target for elucidating the mechanistic model of diabetes, and this starts with glucose uptake. While measurements of cellular/islet glucose transport have been demonstrated in fundamental research using biosensors, the sensitivity and reliability have limited broad application of such an important and powerful tool in the fundamental research domain. Nanotechnology-inspired biosensors have shown promising results—displaying the potential necessary for highly sensitive glucose detection. In the cellular research domain, critical performance criteria are sensitivity and limit of detection.

Nanomaterials such as carbon nanotubes (CNTs) and metallic nanoparticles exhibit impressive results in electrochemical glucose biosensing.[8,9] Nano-inspired electrochemical biosensors are advantageous because of their low power requirements (<1 V), fast response times (<10 s), and high sensitivities (<1 mM).[10] Carbon nanotubes in particular boast exceptional electrochemical glucose sensing capabilities through their inherent ability to catalyze a variety of redox reactions with hydrogen peroxide ($H_2O_2$) and nicotinamide adenine dinucleotide (NADH),[11] the two chemical products typically measured during enzymatic glucose sensing. Conventional electrode materials such as platinum (Pt),[12] palladium,[13] and gold[14] have been scaled down and combined with CNT-based glucose biosensors to enhance electrochemical performance and facilitate enzyme immobilization. CNT/metallic nanoparticle composite glucose biosensors have displayed impressive results—demonstrating high sensitivities and low detection limits necessary for highly accurate and precise blood glucose sensing. Despite these promising results, several technical barriers, including complex biofunctionalization strategies, prevent nanotechnology-inspired biosensors from developing into autonomous glucose monitoring systems.

In this article, we present a CNT/nanoparticle composite glucose biosensor that is shown to facilitate sensitive glucose sensing while avoiding the complex fabrication schemes that have hindered the development of nano-inspired glucose biosensors. We introduce a bottom-up approach to CNT growth combined with metallic nanoparticle deposition and a simple drop-coat bio-functionalization scheme for glucose monitoring—methods that can be easily scaled for commercial production. Results from this new CNT-based biosensor are discussed and correlated to the enhanced electrocatalytic, mass-transport, and enzyme-coupling characteristics of the developed biosensor.

## Methods

The CNT biosensor is fabricated *in situ* from an oxidized silicon wafer [P <100> Si (5 μm), $SiO_2$ (500 nm)] upon which a porous anodic alumina (PAA) template is created for subsequent CNT growth by a method previously reported.[14,15] This PAA template is created by consecutively evaporating a thin metal film stack consisting of titanium (100 nm), aluminum (100 nm), iron (1 nm), and aluminum (400 nm). The metalized silicon wafer is anodized by biasing the substrate to 40 V relative to a Pt auxiliary electrode within an oxalic bath (0.3 M, 5 °C). This anodization process not only forms semi-ordered pores that extend through the aluminum and iron layers to the underlying titanium layer but

also creates a corrosion-resistant surface that decreases the extent of chemical fouling during electrochemical biosensing. A portion of the substrate is left unanodized, leaving an electrically addressable metal contact pad for subsequent electrochemical processing and for possible electronic integration with portable glucose monitoring systems.

Carbon nanotube synthesis initiates within individual PAA pores by a microwave plasma chemical vapor deposition (MPCVD) process (SEKI $A_X$5200S). Within the MPCVD reaction chamber, the anodized substrate is heated to 900 °C under a 10 torr hydrogen ambient by a 3.5 kW radio-frequency power supply. A 300 W unbiased hydrogen plasma is subsequently formed over the substrate, and methane gas is introduced to accomplish CNT growth. The CNTs grow from the iron catalyst layer embedded within the pores of the PAA and extend horizontally 3–10 μm in length along the surface of the substrate. Platinum from a 15 mM $H_2PtCl_6$ salt solution is subsequently electrodeposited onto the substrate through a three-electrode electrochemical setup (BASi Epsilon Three-Electrode Cell Stand), where the CNT/PAA substrate acts as the working electrode, platinum gauze as the auxiliary electrode, and Ag/AgCl (3 M NaCl) as the reference electrode. Through this electrodeposition process, platinum first partially fills the pores of the PAA, creating an electrical contact between the CNTs and conducting titanium bottom layer. Once the Pt wires reach the bases of the CNTs within the pores, the contacted CNTs become part of the electrode, allowing Pt nanospheres (approximately 200 nm in diameter, see **Figure 1**) to form concentrically at defect sites on the portions of the CNTs extending out of the pores.

In order to verify the biofunctionalization compatibility via physical adsorption, a CNT electrode is first checked for autofluorescence and then exposed to fluourescein isothiocyanate (FITC)-labeled bovine serum albumin (BSA);(Sigma-Aldrich, Catalog # A-9771) and fluorescently analyzed again by a Nikon Labophot fluorescence microscope with a FITC filer and an Optronics 479T charge-coupled device camera. The FITC-labeled BSA is exposed to the CNT electrode by first immersing the electrode with phosphate buffer saline (PBS) within an individual polystyrene well. A 5 μl solution of FITC-labeled BSA (2 mg/ml in PBS) is injected into the well and allowed to incubate at room temperature by placing the well on a rotary shaker (100 rpm for 30 min). The CNT electrode is then washed three consecutive times by injecting the well with fresh PBS and agitating it with the rotary shaker (100 rpm for 5 min).
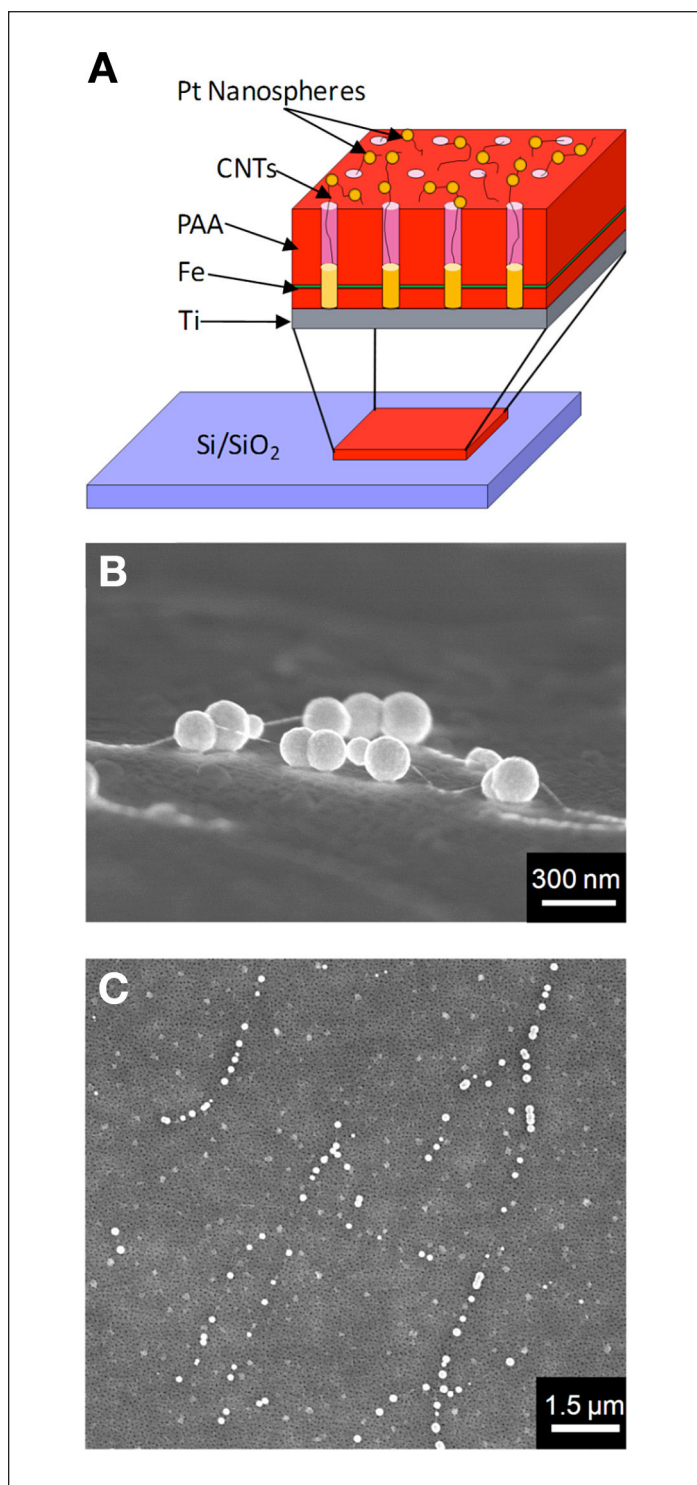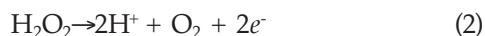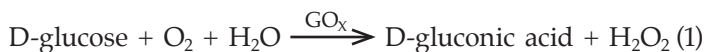


**Figure 1.** Cross-sectional schematic portraying **(A)** the templated *in situ* growth of CNTs from the PAA decorated with Pt nanospheres to form the CNT/Pt nanosphere electrode on an oxidized silicon wafer. Field emission electron microscopy micrographs show **(B)** a high magnified side view and **(C)** low magnified top view of Pt nanospheres (~200 nm diameter) electrically wired by CNTs (~1–3 nm diameter).

This rigorous washing scheme helps to minimize nonspecific binding by eliminating any unbound FITC-labeled BSA from the CNT surface. The washed CNT

electrode is analyzed with fluorescence microscopy by placing the electrode into a transparent PBS-filled chamber that is created by sandwiching a silicone gasket between two glass microscope slides. Adsorbed FITC-labeled BSA is observed on the biofunctionalized CNT electrode via fluorescence microscopy with exposure times of 500 ms and 1 s, respectively.

Using the localization techniques similar to those employed in the foregoing fluorescently marked BSA adsorption experiments, the CNT/Pt nanosphere electrode is converted into a glucose biosensor by immobilizing the enzyme glucose oxidase ($GO_X$) to the electroactive surface of the biosensor via a cross-linking matrix of BSA and glutaraldehyde. Glucose oxidase solution is prepared from a 130,000 U/gm stock (Sigma Aldrich, St. Louis, MO) by dissolving 25 mg of $GO_X$ in 1 ml of PBS (pH 7.4). The enzyme matrix is then created by mixing $GO_X$ solution with 2.5% BSA and 2.5 % glutaraldehyde. The immobilization scheme is based on covalent linking of the enzyme with the aldehyde groups of glutaraldehyde through formation of Schiff bases. Bovine serum albumin serves to block the excess aldehyde sites that can denature the enzyme and aids in maximizing enzyme activity. A 5 µl drop of this enzyme matrix solution (16.25 U $GO_X$) is uniformly pipetted onto the sensor surface and allowed to air dry for 1 h before electrochemical testing. When not in use, the biosensor is stored in PBS (pH 7.4) at 4 °C.

Amperometric $H_2O_2$ and glucose biosensing is carried out through a three-electrode arrangement in which the $GO_X$–CNT/Pt nanosphere biosensor acts as the working electrode, a Pt wire as the auxiliary, and Ag/AgCl as the reference. The three electrodes are immersed in a vial containing 20 ml of PBS (pH 7.4) that is continuously stirred with a 1 cm (length) magnetic bar that rotates at 500 rpm, while successive concentration increases of $H_2O_2$ or D glucose are injected into the test vial at a working potential of 350 mV. The enzymatic reaction of $GO_X$ and D-glucose produces $H_2O_2$ [see **Equations (1)** and **(2)**], which is subsequently oxidized at the electrochemically active CNT/Pt nanosphere surface—producing a measurable electrical signal.

$$\text{D-glucose} + O_2 + H_2O \xrightarrow{\;GO_X\;} \text{D-gluconic acid} + H_2O_2 \quad (1)$$

$$H_2O_2 \rightarrow 2H^+ + O_2 + 2e^- \quad\quad (2)$$

# Results

## Fluorescence Microscopy

In order to demonstrate the biofunctionalization capability of nonchemically altered MPCVD-grown CNT electrodes, fluorescence microscopy is employed to analyze CNT protein adsorption. Fluorescence microscopy results (see **Figure 2**) reveal the relative intensity peaks of the CNT/PAA electrodes with and without immobilized FITC-labeled BSA protein.

The fluorescence intensity with immobilized BSA protein (~0.5 k) is five times greater in intensity than the intensity peak associated with the CNT-only electrode (~0.1 k). The fluorescence micrograph (inset) portrays the binding of the FITC-labeled BSA protein to the CNT array biosensor. These results are consistent with the scanning electron microscope image patterns of CNT/Pt distribution, a view that is corroborated by previous reports of spontaneous adsorption of protein on CNTs,[16] where surface acidic groups located at CNT defect sites enhance the binding of proteins to the CNT arrays.[17,18] Thus this experiment suggests that the MPCVD-grown CNTs possess an adequate quantity of defect sites created during the high-temperature hydrogen plasma ($H_2$–$CH_4$) CNT growth conditions[19] for subsequent noncovalent CNT/enzyme functionalization and biosensing experimentation.
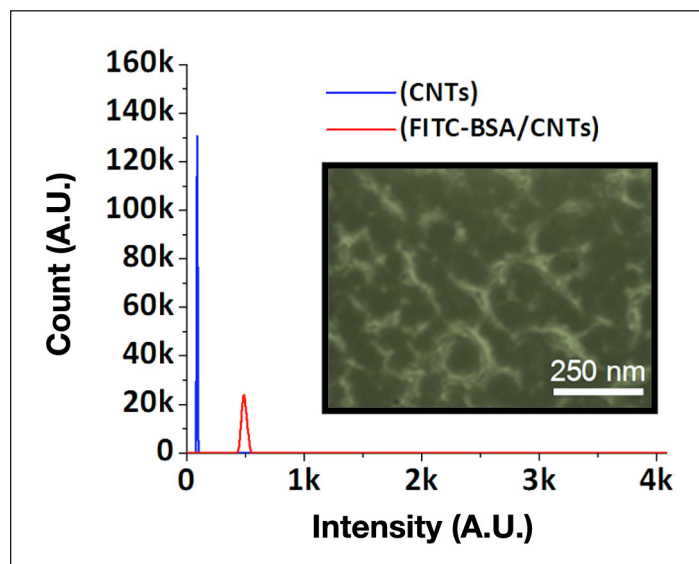


**Figure 2.** Fluorescence intensity results (exposure time = 1 s) for MPCVD-grown CNT electrodes with and without immobilized FITC-labeled BSA. The inset shows a fluorescence micrograph demonstrating the capture of FITC-labeled BSA protein to the CNT array biosensor.

## Hydrogen Peroxide Sensing

Amperometric $H_2O_2$ testing (see **Figure 3**) is performed in order to test the electroactive nature of the of the CNT/Pt nanosphere biosensor toward oxidation of $H_2O_2$. The CNT/Pt nanosphere biosensor exhibits a high sensitivity (7.4 $\mu A/mM/cm^2$) toward the oxidation of $H_2O_2$. This sensitivity is important, as $H_2O_2$ is the electrochemical transducer between the $GO_X$ enzyme and the biosensor surface.
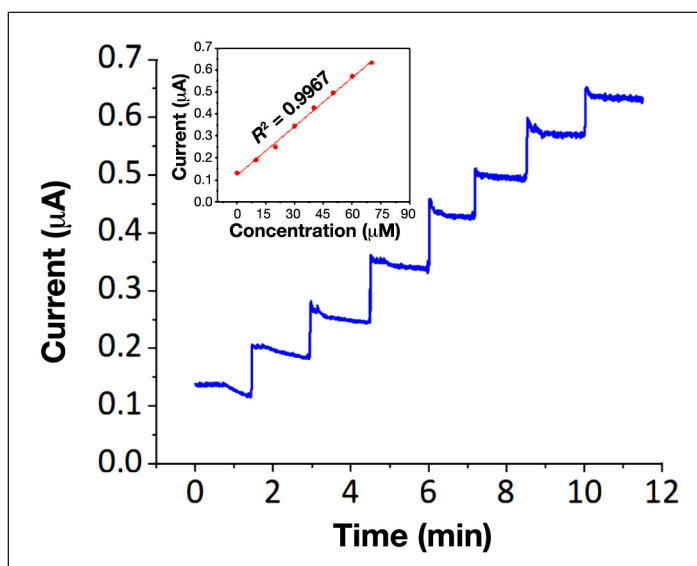


**Figure 3.** Amperometric $H_2O_2$-sensing experiment for the CNT/Pt nanosphere biosensor. The current response to seven successive concentration increases of 10 $\mu M$ $H_2O_2$ is displayed, while the inset shows the linear regression analysis of the current versus concentration plot.

## Glucose Sensing

The $GO_X$ biofunctionalized CNT/Pt nanosphere biosensor was subsequently tested with increasing concentrations of D glucose following the same three-electrode experimental protocol established during $H_2O_2$ sensing (see **Figure 4**).

The micromolar sensitivity of the biosensor is 70 $\mu A/mM/cm^2$, while the response time is 8 s ($t_{90\%}$). In terms of glucose sensitivity and detection limit, the CNT/Pt nanosphere biosensor outperforms CNT/Pt nanoparticle,[20–24] gold nanoparticle,[25] CNT,[26,27] porous carbon,[28] and modified glassy carbon[29–31] amperometric glucose biosensors (see **Table 1**). Furthermore, the $GO_X$–CNT/Pt nanosphere biosensor demonstrates a glucose detection limit of 380 nM at a signal-to-noise ratio of 3, with linear sensing region from 1 $\mu M$ to 0.75 mM (see **Figure 5**).

In order to evaluate the biological activity of immobilized $GO_X$ enzyme on biosensor surfaces, the effective or apparent Michaelis–Menten constant ($K'_M$) is often determined amperometrically from the Lineweaver–Burk-type equation,[32]

$$\frac{1}{i_{ss}} = \left(\frac{K'_M}{i_{max}}\right)\left(\frac{1}{C}\right) + \left(\frac{1}{i_{max}}\right) \tag{3}$$

where $i_{ss}$ is the steady state current associated with a given concentration ($C$) and $i_{max}$ is the measured current during analyte saturation. The apparent
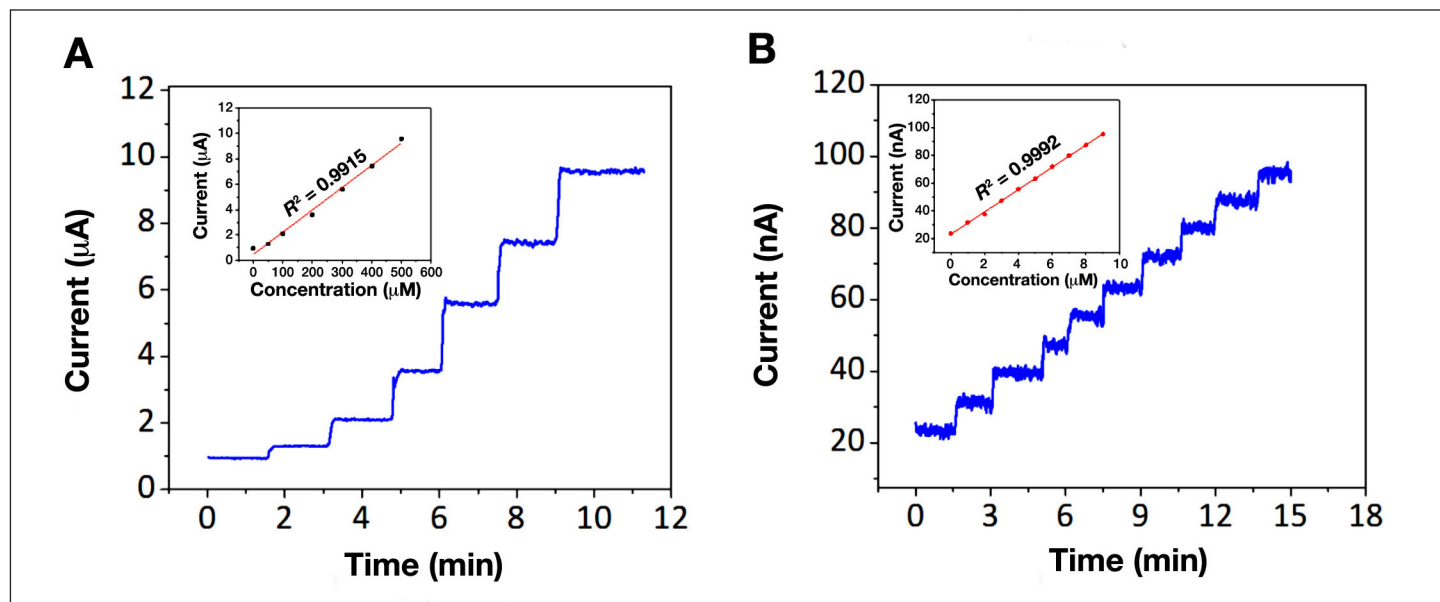


**Figure 4.** Amperometric glucose calibration experiments for the CNT/Pt nanosphere biosensor. The micromolar linear range calibration plot **(A)** portrays the current response to concentration increases of 50, 100, 200, 300, 400, and 500 $\mu M$, while the experimental detection limit graph **(B)** portrays the current response to eight successive concentration increases of 1 $\mu M$, respectively. Insets display the linear regression analysis of the respective current versus concentration plots.

Michaelis–Menten constant of the CNT/Pt nanosphere biosensor is calculated to be 0.64 mM by analyzing the slope and intercept of the Lineweaver–Burk equation plot (see **Figure 5**). This apparent Michaelis–Menten constant is lower than 2.6 mM reported for a similar CNT/Pt-nanoparticle-based biosensor,[22] 8.2 mM of a CNT-based biosensor,[27] 22 and 23 mM reported by more traditional $GO_X$/glucose biosensors derived from solgel and glassy carbon materials,[31,33,34] and lower than 33 mM reported for $GO_X$ in solution phase.[35] Assuming that the rate of the enzymatic reaction is catalysis controlled,[32] the lower apparent Michaelis–Menten constant does correlate with our sensitivity and limit of detection, suggesting that the CNT/Pt nanosphere biosensor creates a unique nanoenvironment for enzymatic biosensing.

## Conclusions

The advantages of utilizing CNTs and Pt nanoparticles over conventional materials in electrochemical glucose biosensing include increased effective surface area, enhanced mass transport of target analytes to what is effectively functional as nanoelectrodes arrays, and improved catalysis as redox transducers.[9,36] The $GO_X$–CNT/Pt nanosphere biosensor reported here achieved a glucose detection limit (380 nM) that was significantly lower than the glucose detection limit (1.3 µM) of our previous CNT-based biosensor that utilized gold-coated palladium nanocubes connected through CNT networks.[14] We hypothesize that the low detection limit and high glucose sensitivity of the $GO_X$–CNT/Pt nanosphere biosensor is related to the electrocatalytic properties of the CNT/Pt nanosphere networks, the high $GO_X$ enzyme activity, and the enhanced electroanalytical mass transport created by individual CNT/Pt nanoelectrodes arrays.

We believe that the key to the enhanced mass transport to the $GO_X$–CNT/Pt nanosphere biosensor is related to the unique nanoenvironment created by the electrically connected networks of CNTs and Pt nanospheres. The Pt-nanosphere-decorated arrays of CNTs are spatially distanced by approximately 2–20 µm on the surface of the electrically insulating PAA (see **Figure 1**). This average spatial distance of at least 10 times the diameter of the individual Pt nanospheres ensures radial diffusion of glucose to the conducting CNT/Pt nanosphere networks on the planar biosensor surface.[37] Radial diffusion to arrays of individual conducting nanoelectrodes situated on planar biosensor surfaces significantly enhance the sensitivity and signal-to-noise ratio during electrochemical biosensing.[38,39]

**Table 1.**
**Glucose-Sensing Performance Comparison between Carbon- and Metallic-Nanoparticle-Based Biosensors**[a]

| Biosensor Description | Sensitivity (µA/mM/cm²) | Detection Limit (µM) | Reference |
|---|---|---|---|
| $GO_X$-glutaraldehyde/Pt-SWCNTs/PAA | 70 | 0.38 | this work |
| $GO_X$/PtNW-CNTs-chitosan/GCE | 30 | 3 | 21 |
| $GO_X$/Pt-CNTs/TiO₂ | 0.24 | 6 | 20 |
| $GO_X$-glutaraldehyde/Pt-MWCNTs/GCE | 52.7 | 30 | 23 |
| $GO_X$-Pt-(solgel)/MWCTNs/CPE | 0.98 | — | 24 |
| $GO_X$-Nafion-Pt-CNTs/GCE | — | 55 | 22 |
| Nafion/$GO_X$-GNPs/GCE | 6.5 | 34 | 25 |
| $GO_X$/ASWCNTs/Si | — | 80 | 26 |
| $GO_X$/CNTs-chitosan/GCE | 0.52[b] | — | 27 |
| Poly-electrolyte-$GO_X$/PCE | 4.39[b] | 10 | 28 |
| $GO_X$-silica-(solgel)/CE | 2.42[b] | 40 | 30 |
| $GO_X$-iridium/GCE | 0.46[b] | 370 | 31 |

[a] The dashes signify that the value was not reported in the corresponding reference. SWCNT, single-walled carbon nanotube; PtNW, platinum nanowire; GCE, glassy carbon electrode; MWCNT, multi-walled carbon nanotube; CPE, carbon paste electrode; GNP, gold nanoparticle; ASWCNT, aligned single-walled carbon nanotubes; PCE, porous carbon electrode; CE, carbon electrode.
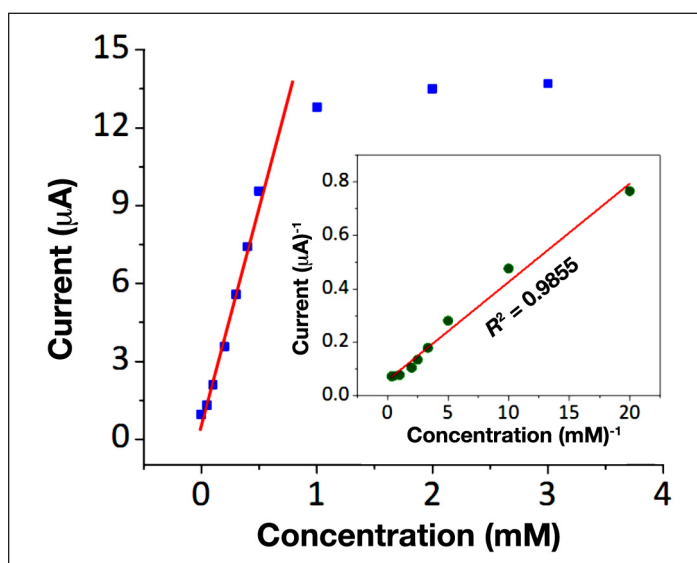[b] The sensitivity was not normalized on a per-unit-area basis.



**Figure 5.** Amperometric glucose calibration plot of the CNT/Pt nanosphere biosensor. The inset portrays the plot of the Lineweaver–Burk equation with linear regression analysis.

This enhanced mass transport of glucose to the biosensor surface is due to the three-dimensional spherical growth of the diffusion boundary layer around the individual Pt nanospheres, which are separated by nonconducting oxide on the planar surface. This is in contrast with conventional planar-surface-based biosensors, which are limited to two-dimensional planar diffusion boundary layer growth that impedes the mass transfer of glucose to the biosensor surface. This interpretation is based on an extension of Bard and Faulkner's fundamental model[40] that explains the enhanced performance of microelectrodes over planar macroelectrodes.

Additional characteristics that enhance biosensor performance are the electrocatalytic properties and high surface area of the nanostructured Pt nanospheres and CNTs. These biosensor attributes potentially enhance the charge transport characteristics of the biosensor. We believe that the unique nanoenvironment of the nanostructured surface of the biosensor is well suited to preserve the tertiary structure of the $GO_X$ enzyme—thus permitting a high enzyme activity. We hypothesize that all these characteristics, including the mass transport, electroactive, and enzymatic properties of the $GO_X$–CNT/Pt nanospheres, combine synergistically to create a biosensor capable of sensing glucose at submicromolar concentrations.

One significant area of emphasis in our program is on the development of tools for fundamental physiology research. For these applications, the submicromolar glucose sensitivity of the $GO_X$–CNT/Pt nanosphere biosensor is well suited for monitoring the glucose transport process in pancreatic β cells, via a self-referencing microbiosensor.[41] In these cell physiology research applications, high glucose sensitivity significantly enhances the spatial and temporal resolution of the biosensor[41,42] at the levels of the cellular/subcellular domain. Because of the mechanistic relationship between glucose uptake, cell signaling, and insulin secretion, measuring glucose flux across cellular membranes is crucial to understanding diabetes. Ultimately, regulation of insulin release from pancreatic islets and β cells depends in part on glucose uptake.[7,43]

In summary, the $GO_X$–CNT/Pt nanosphere biosensor outperforms similar Pt nanoparticle/CNT biosensors in terms of glucose sensitivity and detection limit. The CNT/Pt nanosphere biosensor demonstrates a glucose sensitivity of 70 μA/mM/cm², low detection limit 380 nM (signal-to-noise ratio = 3), and linear sensing region from 1 μM to 0.75 mM. Although the

$GO_X$–CNT/Pt nanosphere biosensor is currently well suited for cell physiology research, we also believe it can be utilized for blood glucose sensing. Based on this platform and our experience from previous work,[14] we believe that altercation of the enzyme immobilization scheme will allow us to engineer the sensor for linear glucose sensing within the physiological range for blood glucose (3.8–6.1 mM).[31] The high enzyme activity of the immobilized $GO_X$ enzyme, the enhanced mass transport (glucose and/or $H_2O_2$), and the efficient redox activities associated with the CNT/Pt nanospheres are key reasons for the glucose sensing results reported here.

In terms of commercial production, the *in situ* fabrication protocol of the biosensor eliminates the need for exhaustive CNT and Pt nanoparticle processing steps. Thus the facile biosensor fabrication and biofunctionalization scheme of the $GO_X$–CNT/Pt nanosphere biosensor can be scaled easily for mass production of research-grade microsensors or future integration into commercial glucose monitoring systems that require highly sensitive and accurate glucose sensing.

**References:**

1. Baliga BS, Weinberger J. Diabetes and stroke: part one—risk factors and pathophysiology. Curr Cardiol Rep. 2006;8(1):23–8.

2. Chuang H, Trieu MQ, Hurley J, Taylor EJ, England MR, Nasraway SA Jr. Pilot studies of transdermal continuous glucose measurement in outpatient diabetic patients and in patients during and after cardiac surgery. J Diabetes Sci Technol. 2008;2(4):595–602.

3. Van den Berghe G, Wouters P, Weekers F, Verwaest C, Bruyninckx F, Schetz M, Vlasselaers D, Ferdinande P, Lauwers P, Bouillon R. Intensive insulin therapy in critically ill patients. N Engl J Med. 2001;345(19):1359–67.

4. Harris G. Standards might rise on monitors for diabetics. The New York Times. July 18, 2009.

5. Kahn BB. Facilitative glucose transporters: regulatory mechanisms and dysregulation in diabetes. J Clin Invest. 1992;89(5):1367–74.

6. Meglasson MD, Matschinsky FM. Pancreatic islet glucose metabolism and regulation of insulin secretion. Diabetes Metab Rev. 1986;2(3-4):163–214.

7. Unger RH. Diabetic hyperglycemia: link to impaired glucose transport in pancreatic beta cells. Science. 1991;251(4998):1200–5.

8. Wang J. Nanomaterial-based electrochemical biosensors. Analyst. 2005;130(4):421–6.

9. Welch CM, Compton RG. The use of nanoparticles in electroanalysis: a review. Anal Bioanal Chem. 2006;384(3):601–19.

10. Wang J. Carbon-nanotube based electrochemical biosensors: a review. Electroanalysis. 2005;17(1):7–14.

11. Gooding JJ, Wibowo R, Liu J, Yang W, Losic D, Orbons S, Mearns FJ, Shapter JG, Hibbert DB. Protein electrochemistry using aligned carbon nanotube arrays. J Am Chem Soc. 2003;125(30):9006–7.

12. Hrapovic S, Liu Y, Male KB, Luong JH. Electrochemical biosensing platforms using platinum nanoparticles and carbon nanotubes. Anal Chem. 2004;76(4):1083–8.

13. Lim SH, Wei J, Lin J, Li Q, Kuayou J. A glucose biosensor based on electrodeposition of palladium nanoparticles and glucose oxidase onto Nafion-solubilized carbon nanotube electrode. Biosens Bioelectron. 2005;20(11):2341–6.

14. Claussen JC, Franklin AD, Ul Haque A, Porterfield DM, Fisher TS. Electrochemical biosensor of nanocube-augmented carbon nanotube networks. ACS Nano. 2009;3(1):37–44.

15. Franklin AD, Janes DB, Claussen JC, Fisher TS, Sands TD. Independently addressable fields of porous anodic alumina embedded in SiO2 on Si. Appl Phys Lett. 2008;92:013122.

16. Guiseppi-Elie A, Lei C, Baughman RH. Direct electron transfer of glucose oxidase on carbon nanotubes. Nanotechnology. 2002;13(5):559–64.

17. Hirsch A. Functionalization of single-walled carbon nanotubes. Angew Chem Int Ed. 2002;41(11):1853–9.

18. Banks CE, Compton RG. New electrodes for old: from carbon nanotubes to edge plane pyrolytic graphite. Analyst. 2006;131(1):15–21.

19. Maschmann MR, Amama PB, Goyal A, Iqbal Z, Gat R, Fisher TS. Parametric study of synthesis conditions in plasma-enhanced CVD of high-quality single-walled carbon nanotubes. Carbon. 2006;44(1):10–8.

20. Pang X, He D, Luo S, Cai Q. An amperometric glucose biosensor fabricated with Pt nanoparticle-decorated carbon nanotubes/TiO2 nanotube arrays composite. Sens Actuators B. 2009;137(1):134–8.

21. Qu F, Yang M, Shen G, Yu R. Electrochemical biosensing utilizing synergic action of carbon nanotubes and platinum nanowires prepared by template synthesis. Biosens Bioelectron. 2007;22(8):1749–55.

22. Wen Z, Ci S, Li J. Pt nanoparticles inserting in carbon nanotube arrays: nanocomposites for glucose biosensors. J Phys Chem C. 2009;113(31):13482–7.

23. Xie J, Wang S, Aryasomayajula L, Varadan VK. Platinum decorated carbon nanotubes for highly sensitive amperometric glucose sensing. Nanotech. 2007;18(6):65503–601.

24. Yang M, Yang Y, Liu Y, Shen G, Yu R. Platinum nanoparticles-doped sol-gel/carbon nanotubes composite electrochemical sensors and biosensors. Biosens Bioelectron. 2006;21(7):1125–31.

25. Zhao S, Zhang K, Bai Y, Yang W, Sun C. Glucose oxidase/colloidal gold nanoparticles immobilized in Nafion film on glassy carbon electrode: direct electron transfer and electrocatalysis. Bioelectrochemistry. 2006;69(2):158–63.

26. Lin Y, Lu F, Tu Y, Ren Z. Glucose biosensors based on carbon nanotube nanoelectrode ensembles. Nano Lett. 2004;4(2):191–5.

27. Liu Y, Wang M, Zhao F, Xu Z, Dong S. The direct electron transfer of glucose oxidase and glucose biosensor based on carbon nanotubes/chitosan matrix. Biosens Bioelectron. 2005;21(6):984–8.

28. Gavalas VG, Chaniotakis NA. Polyelectrolyte stabilized oxidase based biosensors: effect of diethylaminoethyl-dextran on the stabilization of glucose and lactate oxidases into porous conductive carbon. Anal Chim Acta. 2000;404(1):67–73.

29. Gouveia-Caridade C, Pauliukaite R, Brett CM. Development of electrochemical oxidase biosensors based on carbon nanotube-modified carbon film electrodes for glucose and ethanol. Electrochim Acta. 2008;53(23):6732–9.

30. Li J, Chia LS, Goh NK, Tan SN. Renewable silica sol-gel derived carbon composite based glucose biosensor. J Electroanal Chem. 1999;460(1-2):234–41.

31. Rodríguez MC, Rivas GA. Glucose biosensor prepared by the deposition of iridium and glucose oxidase on glassy carbon transducer. Electroanalysis. 1999;11(8):558–64.

32. Kamin RA, Wilson GS. Rotating ring-disk enzyme electrode for biocatalysis kinetic studies and characterization of the immobilized enzyme layer. Anal Chem. 1980;52(8):1198–205.

33. Sampath S, Lev O. Inert metal-modified, composite ceramic–carbon, amperometric biosensors: renewable, controlled reactive layer. Anal Chem. 1996;68(13):2015–21.

34. Wang B, Li B, Deng Q, Dong S. Amperometric glucose biosensor based on sol-gel organic-inorganic hybrid material. Anal Chem. 1998;70(15):3170–4.

35. Swoboda BE, Massey V. Purification and properties of the glucose oxidase from aspergillus niger. J Biol Chem. 1965;240(5):2209–15.

36. Katz E, Willner I, Wang J. Electroanalytical and bioelectroanalytical systems based on metal and semiconductor nanoparticles. Electroanalysis. 2004;16(1-2):19–44.

37. Fletcher S, Horne MD. Random assemblies of microelectrodes (RAM™ electrodes) for electrochemical studies. Electrochem Commun. 1999;1(10):502–12.

38. Arrigan DW. Nanoelectrodes, nanoelectrode arrays and their applications. Analyst. 2004;129(12):1157–65.

39. Compton RG, Wildgoose GG, Rees NV, Streeter I, Baron R. Design, fabrication, characterisation and application of nanoelectrode arrays. Chem Phys Lett. 2008;459(1-6):1–17.

40. Bard AJ, Faulkner LR. Electrochemical methods: fundamentals and applications. Wiley: New York; 1980.

41. Porterfield DM. Measuring metabolism and biophysical flux in the tissue, cellular and sub-cellular domains: Recent developments in self-referencing amperometry for physiological sensing. Biosens Bioelectron. 2007;22(7):1186–96.

42. Jung SK, Trimarchi JR, Sanger RH, Smith PJ. Development and application of a self-referencing glucose microsensor for the measurement of glucose consumption by pancreatic beta-cells. Anal Chem. 2001;73(15):3759–67.

43. Schuit FC, Huypens P, Heimberg H, Pipeleers DG. Glucose sensing in pancreatic beta-cells: a model for the study of other glucose-regulated cells in gut, pancreas, and hypothalamus. Diabetes. 2001;50(1):1–11.