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# Fiber optic biosensor for transdermal glucose based on the glucose binding protein

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ABSTRACT

In previous work, a painless, noninvasive method of collecting transdermal glucose (TG) passively diffusing through the skin was developed. The transdermal glucose collected in this way has concentrations in the μM range requiring a reliable sensor that can measure glucose at these levels. In this study, a fiber optic biosensor for μM glucose based on the glucose binding protein (GBP) is described. The GBP was labeled with BADAN in the H152C position and immobilized on Ni-NTA agarose beads via metal-histidine interaction. The portable, low-cost biosensor system consists of an optical fiber with the sensitized beads trapped on one end, and appropriate optics and electronics on the other end. The control software and the visual interface for the optical sensor is designed and implemented in LabVIEW and runs on a tablet computer. Optimization of sensor response was performed by varying the amount of Ni-NTA-GBP sensitized beads and its distance to the optical fiber. The biosensor exhibited a stable response to buffer and in 10.0 μM glucose even after ~16 h of continuous use. Glucose response is reversible when cycled between phosphate-buffered saline solution and various glucose solutions. The response time of the biosensor in 6 μM glucose was approximately 50 s. A linear relationship ($r^2 = 0.990$) between sensor response and glucose concentrations from 4.00 to 20.00 μM was obtained. Measurement of transdermal glucose diffusion was demonstrated with Yucatan minipig skin as surrogate for human skin. The results show the potential of this fiber optic biosensor as a transdermal glucose monitoring system at the point of care.



## 1. Introduction

The measurement of glucose levels is a critical part of good clinical care not only in diabetic patients but also in premature neonates and in critically ill patients. The current standard of care for obtaining glucose levels – point-of-care glucometers or bedside and laboratory glucose analyzers – require breaking the skin and collecting a sample of blood from the patient. In recent years, transcutaneous continuous glucose monitoring systems (CGMS) have gained acceptability. These methods are painful and messy and limits compliance in adult patients. For neonates, breaking the skin cause pain with short and long-term consequences, potential exposure to infectious agents and other complications. Noninvasive monitoring of glucose can facilitate a more effective management of hyperglycemic and hypoglycemic episodes without the adverse effects of current technology.

Different physiological fluids have been used as matrices for noninvasive methods of measuring glucose levels in the body. These include urine [1,2], sweat [3], saliva [4–6] and tears [7–10]. Lag time and complexity of mixtures are issues associated with these matrices.

Transdermal glucose has also been previously reported for minimally invasive glucose measurement. Because TG concentrations are $10^3$–$10^4$ times less than in blood, extraction methods such as by iontophoresis [11,12], heat [13], physical abrasion, vacuum application or ultrasound are necessary to allow for detection by currently available enzyme-based glucose sensors. The GlucoWatch™, which utilized iontophoresis for TG extraction, was

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approved by the FDA but proved to be too expensive for the target market.

In contrast to enzyme-based glucose sensors, our group has been developing an extremely sensitive non-enzymatic glucose biosensor based on the *E. coli* glucose binding protein (GBP) [14–17]. Using this sensor, we have shown detection of TG in both neonates and adults by passive diffusion of glucose through skin. Passive diffusion involves the spontaneous transfer of glucose through the skin. It does not require breaking the skin or the application of chemical or physical agents. It does not cause pain, exposure to potential infection or foreign body response (as in a transcutaneously embedded needle). A strong correlation between TG and blood glucose was found, which indicates the feasibility of this method for noninvasive sensing of glucose [18]. Because TG is 3–4 orders of magnitude less concentrated than blood glucose, it is too dilute to be detected by conventional enzyme-based glucose sensors. The sensitivity of GBP falls exactly within this range, making it ideal for detecting these trace amounts.

The TG extracted by diffusion is directly measured by fluorescently-labeled GBP. Our group and others have taken advantage of the conformational changes in GBP as it binds to glucose in designing the glucose biosensor [14,15,19–23]. A polarity-sensitive fluorescent probe, such as BADAN (6-bromoacetyl-2-dimethylaminonaphthalene), attached to a cysteine mutation on the GBP provides the signal to distinguish the glucose-free and glucose-bound conformations. The first to report the H152C position as a suitable site for fluorescence labeling was De Lorimier, et al. [19]. This was followed by Amiss et al. [20] who immobilized the GBP-H152C-BADAN with a $(His)_6$-tag to metal ligand chromatography beads. Saxl et al. reported the development of a fiber optic biosensor using the GBP-H152C-BADAN immobilized on Ni-NTA microbeads [24]. The efforts of these groups were primarily to increase the $K_d$ of the GBP to match the mM concentration of glucose in blood and interstitial fluid. For their purposes, the GBP-H152C-BADAN is a stepping stone for further genetic engineering towards a transcutaneous biosensor with a $K_d$ in the mM range [25]. A GBP with multiple mutations has been tested in both preclinical [21,26,27] and clinical [25] studies of transcutaneous glucose measurements.

In contrast, the aim of this paper is to describe the development of the single-site mutant GBP-H152C-BADAN fiber optic biosensor and its potential to measure TG at the point of care. The difference between our group and the others who worked on the same protein sensor is the advancement of a total glucose sensing system (fiber optic biosensor and minifluorimeter) that is low-cost, low-power, accurate and easy to use. In addition, we show *in vitro* data on Yucatan minipig skin mounted on a Franz cell to illustrate the feasibility of this glucose sensing system to directly measure passive diffusion of TG through skin. Our immediate objective is to use this noninvasive TG monitoring system in the neonatal intensive care unit.

## 2. Experimental

### 2.1. Materials

D-Glucose, $NaH_2PO_4$, $Na_2HPO_4$ and Coomassie Brilliant Blue solution were purchased from Sigma-Aldrich (St. Louis, MO). Isopropyl-2-D-thiogalactopyranoside (IPTG), Tris-hydrochloride and urea were from Fisher Scientific. BADAN (6-bromoacetyl-2-dimethylaminonaphthalene) was obtained from Molecular Probes (Eugene, OR). Sephadex G-25 fine were from GE Healthcare (Bio Sciences, AB).

His Pur Ni-NTA agarose beads (45–160 μm diameter) and tris(2-carboxyethyl) phosphine (TCEP) were obtained from Thermo Scientific. Hydrophilic nylon mesh (20 μm pore size) was obtained from Spectrum Labs (Rancho Dominguez, CA).

### 2.2. Biosensor

GBP H152C was overexpressed in *E. coli* by induction with IPTG. Bacterial cells were lysed using a probe sonicator (Fisher Scientific Sonic Dismembrator Model 500) and the clarified cell extract was purified using affinity chromatography Ni-NTA agarose column. GBP H152C was washed and eluted with buffers containing urea, Tris-HCl and $Na_2HPO_4.H_2O$.

The total protein concentration of the isolated protein was determined using Bradford Protein Assay. The highly pure fractions were dialysed using 20 mM phosphate ($NaH_2PO_4$ and $Na_2HPO_4$) buffer, pH 7.4 at 4 °C.

A 10-fold excess of Tris(2-carboxyethyl)phosphine hydrochloride (TCEP in 10 mg/mL) in deionized water was added to the protein solution to keep thiol groups in the reduced form. BADAN in dimethyl sulfoxide (DMSO) was added to the protein solution and allowed to stand for 2 h at room temperature. Unreacted BADAN was removed from purified protein by size exclusion chromatography, (Sephadex G-25) and by dialysis in 100 mM phosphate ($NaH_2PO_4$ and $Na_2HPO_4$) buffer, pH 7.5. The total protein concentration was determined, and the labeling efficiency was estimated. The final product was 0.22 μm filter-sterilized and stored at 4 °C.

### 2.3. Immobilization of BADAN-labeled GBP to Ni-NTA agarose beads

Forty μL packed Ni-NTA agarose beads were washed three times in PBS (pH 7.4) to remove ethanol. The beads were then incubated with 6.61 mL of GBP-BADAN (10.90 μM) in PBS and mixed in a rotary shaker at 25 rpm for 24 h in the cold room to ensure maximum binding of protein to Ni-NTA agarose beads. The beads were washed, centrifuged, separated from the supernatant and finally re-suspended in 80 μL of PBS, for a total volume of 120 μL. This procedure was carried out based on the reported maximum binding capacity of Ni-NTA agarose beads to protein which is 60 mg protein/mL resin (Thermo Scientific, Waltham, MA). The Ni-NTA-GBP beads in PBS were stored in the refrigerator at 4 °C until use.

### 2.4. Characterization of Ni-NTA-GBP beads

Ni-NTA-GBP agarose beads (about 120 μL in PBS) were placed in a 96-well plate and the fluorescence was measured on a SpectraMax M5 plate reader (Molecular Devices, Sunnyvale, CA). Five μL of 1 M glucose solution was added to the beads to a final glucose concentration of 42 mM and the spectrum was again measured. In estimating the binding constant and maximum glucose binding of the protein, 5 μL of standard glucose solutions were added to the 120 μL of Ni-NTA-GBP agarose beads in PBS and the fluorescence intensities were measured after each addition. The plate was gently shaken for 5 s, and the intensities were measured 4 more times. All measurements were made following the same instrumental conditions: excitation wavelength 400 nm, emission wavelength 550 nm, excitation slit width 5 nm, emission slit width 5 nm, PMT detector voltage 750 V, and average time 0.1 s.

### 2.5. Estimation of binding constant and maximum glucose binding

The binding constant $K_d$, and the maximum glucose binding were calculated by fitting the data of normalized fluorescence intensities at different glucose concentrations to binding-saturation curves using Prism 6 (GraphPad Software, San Diego,