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Portable system for the detection of micromolar concentrations of glucose

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Abstract

Glucose in non-invasively collected biofluids is generally in the micromolar range and thus, requires sensing methodologies capable of measuring glucose at these levels. Here, we present a small fluorometer system that can quantify glucose in the range of 0–5 μM with resolution of $\sim 0.07 \mu\text{M}$. It relies on the glucose binding protein (GBP) fluorescently labeled with two fluorophores. Fluorescence signals from the dual-labeled GBP are utilized in a ratiometric mode, making the measurements insensitive to variations in protein concentration and other systematic errors. Fluorescence is quantified by a miniature, dedicated ratiometric fluorometer that is powered via USB. Concentration is calculated using an ultra-mobile personal computer (UMPC). The whole system is designed to be pocket sized suitable for point-of-care or bedside applications. Test results suggest that the system is a promising tool for accurate measurements of low glucose concentrations (0.1–10 μM) in biological samples.

Keywords

Glucose sensor; portable system; fluorescence

Introduction

Accurate monitoring of glucose concentrations is an essential component of biomedical monitoring and diagnosis. Glucose is the major energy source for cells and is thus involved directly or indirectly in many metabolic pathways. The standard medical practice is to measure blood or plasma glucose levels either with a portable glucose meter or with more sophisticated instrumentation in the laboratory. Blood and plasma glucose concentrations range from about 3.0–20 mM spanning the hypoglycemic to hyperglycemic spectrum. There are other biofluids of biomedical interest but with glucose concentrations in the μM range. These are saliva ($\sim 70 \mu\text{M}$ glucose) [1,2], tears ($\sim 200 \mu\text{M}$) [3,4], and sweat ($\sim 120 \mu\text{M}$) [5,6]. Our group recently reported on transdermal glucose that can easily reach 30–50 μM [7]. These sources of glucose have shown some correlation with blood glucose. But the interest rests mainly on being able to collect the samples noninvasively. The ability to measure blood glucose without a needle prick and with minimal or no pain has been the holy grail of diabetes research. The rationale is that without pain and skin disruption, compliance to glucose testing and monitoring would significantly increase. This in turn will lead to better management of diabetes and its complications.

Current technology for glucose sensing uses the glucose oxidase/dehydrogenase biosensor system (hexokinase is also used but not as widely) [8,9,10,11]. These enzyme biosensors work very well in the mM blood/plasma glucose range. Some applications directly measure the fluorescence of the enzyme cofactor [12], while other utilize labeling with quantum dots

[13]. However, as the concentrations approach single μM levels, device design becomes increasingly difficult and not amenable to low-cost and portability.

There are also several non-enzymatic approaches which explore equilibrium reactions. One of the most advanced is the combined use of surface plasmon resonance readout with glucose binding polymer [14–15] which can achieve limit of detection in high micromolar range.

One alternative to the glucose oxidase/dehydrogenase biosensor is a system based on the glucose binding protein (GBP) [16–21]. In gram-negative bacteria, GBP is responsible for detecting and binding to minute amounts of glucose that diffuse into the periplasmic space, then transporting the glucose to a membrane bound receptor for ATP-fueled transport into the cytoplasm or as a cue for chemotaxis. The GBP is highly specific to glucose and is sensitive to μM concentrations. Our group has converted the GBP into a ratiometric fluorescent glucose biosensor [16], which is in the heart of the described system.

As previously mentioned, biofluids collected by noninvasive means require a biosensor that is sensitive in the μM range. The fluorescence based GBP sensor is designed as an alternative to the current glucose oxidase/dehydrogenase sensor system. Our own interest is in measuring transdermal glucose passively collected in a small volume of water sitting on the surface of the skin. We showed that the amount of glucose transported by passive diffusion through the skin follows the trend of blood glucose [7]. We were able to show the feasibility of this method by analyzing the transdermal glucose samples with a GBP assay on a benchtop, lab grade fluorometer. However, for the non-invasive transdermal glucose sensing to become a true alternative to the commercial glucometer, a handheld device that can be used for bedside or home care application is necessary.

There are very few commercially available portable fluorometers. One such example of a minifluorometer is manufactured by Ocean Optics and is somewhat portable, but geared toward laboratory use. It is costly at a price tag of several thousand dollars. Existing lower cost fluorometers (i.e. Turner design) are single excitation - single emission instruments and obviously cannot be used to measure the emission at two wavelengths of a dual-labeled biosensor. These devices are primarily used in water analysis (e. g. measurement of chlorophyll, optical brighteners as contaminants, fluorescent tracers, etc.).

In this paper, we present a USB powered, all-solid-state, hand-held fluorometric system for measurement of low glucose concentrations. It utilizes the dual-labeled GBP as sensor and a dedicated low cost, dual emission microfluorometer. The design, construction and testing of this device is described here.

Theory of operation

The system is based on the use of a dual labeled glucose-binding protein (GBP). The wild type of the protein is responsible for glucose sensing and transport in *E. coli*. In this study, we used a mutant of GBP that has a single cysteine mutation positioned at an allosteric site [16]. The protein is subsequently labeled at the mutation with polarity sensitive fluorophore for signal transduction, as well as with a second dye at the N-terminal for reference. The exact details and the spectroscopic studies of the resulting protein are given in [16]. Briefly, when no glucose is present, the protein assumes an “open” position (Figure 1). The polarity probe, which resides on the other side of the “hinge”, is enclosed in a hydrophobic “pocket”. This results in high intensity of the emission. In presence of glucose, the protein switches to a “closed” position thus exposing the probe to water. Its emission gets quenched (Figure 1, B). The second label is not environmentally sensitive thereby serving as a reference. The higher the concentration of the glucose, the lower the signal from the sensing dye, while the

intensity from the reference dye is relatively constant. The ratio of the emission at the two peaks is used to quantify the glucose concentration. The two dyes are excited on the same wavelength, but they exhibit two distinct emission maxima. When measuring glucose, a solution with known concentration of the protein is mixed with the sample in a cuvette and the fluorescence is read using the developed fluorometer.

Quantitatively the equilibrium reaction between glucose Gl and GBP is described by the equation

where $glGBP$ denote the resulting protein-glucose complex. The concentrations of the free GBP ($[fGBP]$), glucose-protein complex $[glGBP]$ and free glucose $[Gl]$ are related through the association constant K :

$$K = \frac{[glGBP]}{[fGBP] \cdot [Gl]} \quad (2)$$

The total concentration of the glucose $[T_G]$ can be determined as a sum of the free glucose concentration $[Gl]$ and the bound glucose concentration $[glGBP]$. Additionally, the total concentration of GBP will be the sum of the concentrations of the free and bound GBP:

$$[T_G] = [Gl] + [glGBP] \quad (3)$$

$$[T_{GBP}] = [fGBP] + [glGBP] \quad (4)$$

Substituting (2) and (3) and rearranging, we obtain

$$[T_G] = k \cdot \frac{1}{\frac{[T_{GBP}]}{[glGBP]} - 1} + [glGBP], \quad (5)$$

where k is the dissociation constant of the reaction ($k = 1/K$).

The block diagram of the ratiometric fluorometer that is used for quantification is shown in Figure 2. The light from a single LED is used to excite the fluorescence. The sample cuvette is “sandwiched” between two photodetectors equipped with bandpass filters. The intensities corresponding to the emission at 520 and 600 nm are quantified simultaneously and sent to the host computer. There, the ratio of the two intensities R is calculated and stored for analysis. Fluorescence intensity at 600 nm, F^{600} is insensitive to glucose concentrations, while fluorescence intensity at 520 nm, F^{520} , decreases with increasing glucose concentrations. Quantitatively, the fluorescence intensity can be found as

$$F = I \cdot Q \cdot f^\lambda = I_0 \left(1 - 10^{-\varepsilon \cdot l \cdot [C]} \right) \cdot Q \cdot f^\lambda,$$

where I is intensity of the absorbed light, I_0 is the intensity of the excitation, ε is the molar extinction coefficient at the exciting wavelength, l is the layer thickness, $[C]$ is the concentration, Q is the quantum yield, and f^λ is the probability that the emission will occur

at the respective emission wavelength. When the concentration of the fluorescent species is low (i.e. the absorption is <0.05), most of these variables can be lumped in a single coefficient a^λ , yielding

$$F^\lambda = a^\lambda [C] \quad (6)$$

After substituting (5) for the respective species and wavelengths in (3), we obtain

$$[T_G] = k \cdot \frac{R_0 - R}{R - R_{\min}} + [T_{GBP}] \cdot \frac{R_0 - R}{R_0 - R_{\min}} \quad (7)$$

where R_0 is the ratio measured in absence of glucose, $R_{\min}$ is the ratio when the protein is saturated with glucose, $[T_{GBP}]$ is the protein concentration, and R is the actual measured ratio.

For full details of the derivation, the reader is referred to the Supplementary information.

Experimental

Materials

6-Acryloyl-2-dimethylaminonaphthalene (acrylodan) and tris(2-carboxyethyl)phosphine (TCEP) were purchased from Molecular Probes (Eugene, OR). Glucose, DEAE Sephadex A-50, N,N-dimethylformamide (DMF), NaCl, KH_2PO_4 , Na_2HPO_4 , NaH_2PO_4 , and MgCl_2 were purchased from Sigma-Aldrich. Tryptone and yeast extract were obtained from Becton Dickinson (Sparks, MD). All chemicals were used without further purification.

Biosensor

The dual-labeled GBP biosensor was prepared as described in [9,10]. Briefly, modifications to the GBP structure to convert it into a fluorescence-based biosensor involved introducing a cysteine mutation at an allosteric site at the 255 position. The GBP was labeled with the polarity sensitive dye, acrylodan in the S255C position, which then responds to increasing glucose concentrations by decreasing fluorescence intensity [15]. A second dye, a ruthenium metal ligand complex (Ru) was attached to the N-terminal of the protein as a reference, allowing for ratiometric measurements. Ru is a good reference because it is not influenced by the protein conformational changes and its fluorescence lifetime is ~ 200 times longer than acrylodan [15, 16]. The GBP glucose-free and glucose-bound structures and the corresponding fluorescence emission spectra are shown in Figure 1.

Microfluorometer system

The handheld glucose detection system presented in Figure 2 consists of electronics integrated with optics (Figure 2, A); a custom microcuvette to hold both sample and GBP biosensor (Figure 2, B); and an ultra-mobile personal computer to control the system and serve as visual interface with the user (Figure 2, C)

Optics

The dual labeled GBP is excited using a 400 nm LED (UV5TZ-395-15, Bivar, CA) and the resulting emission is filtered using a violet-blue Hoya glass filter B-390 (Edmund Optics). To decrease the coupling of the excitation light with the cuvette walls which may result in unwanted background fluorescence, the beam is shaped using a rectangular aperture to a divergence less than 5 arc degrees. A mirror made of aluminized Mylar sheet was attached

on the wall opposite to the excitation aperture to increase the excitation intensity. In this way, the excitation intensity was effectively doubled.

Emission is detected using photodiodes S12232-01 (Hamamatsu). These are fast PIN photodiodes with a large surface area (14 mm²). They are positioned on both sides of the cuvette in a “sandwich” configuration (Figure 2). The photodiodes are equipped with interference filters which allowed selecting the emission from the assay in the 500–550 (“green band”) and 570–630 (“red band”) nm regions. These bands correspond to the acrylodan and Ru emissions, respectively.

Electronics

The LED was driven by a fast voltage-controlled current source (VCCS) to ensure that the excitation intensity is stable when the LED is on. By delivering a square wave voltage on the VCCS input, the sample is excited with modulated light. The amplitude of the light can be independently varied by a digital-to-analog converter (DAC 7571, Texas Instruments).

Photodiodes were employed to detect the fluorescence intensity by generating current proportional to the intensity of the light that reaches them. Because photodiodes do not exhibit internal amplification like photo multipliers or avalanche photodiodes, the photo current is converted to voltage using an op amp in transimpedance differential configuration thereby ensuring amplification.

The amplifier was calculated to provide a transimpedance gain of 10⁸. A single stage transimpedance amplifier with such gain would be difficult to realize, as it would require a feedback resistor of 100 MOhm. Such high resistances are close to the surface resistance of the printed circuit board, on which the amplifier is mounted, and there is a definite possibility for the leakage currents and the ambient humidity to affect the measurements. Therefore, a two-stage amplification system was employed: first stage is with a 10⁷ transimpedance using OPA 301 (Texas Instruments) and second stage is with additional amplification of 20. The second stage was separated from the first using a blocking capacitor, which prevented the DC offsets from the output of the first stage to be amplified. The maximum amplification was in the band between 5 and 20 kHz. The output of the second stage was digitized using a fast analog-to-digital converter (16 bit ADC, ADS8318, Texas Instruments) with a maximum throughput of 500 kilosamples per second. It is read by a microcontroller (MSP430F2272, Texas Instruments), which performs both the LED control and the timed acquisition of the intensities.

Detection algorithm

The LED was modulated at 10 kHz. Due to the high amplification, the output of the photo amplifier reaches steady state after the LED transition (on to off or vice versa) in about 20 μs; therefore, an ADC conversion is performed approximately 30 μs after the LED transition. 10,000 measurements are made when the LED is on and another 10,000 measurements are made when the LED is off. A running sum is calculated according to the following formula:

$$\text{Intensity} = I_1^{\text{LED on}} - I_1^{\text{LED off}} + I_2^{\text{LED on}} - I_2^{\text{LED off}} \dots + I_{10000}^{\text{LED on}} - I_{10000}^{\text{LED off}} \quad (8)$$

where $I_x^{\text{LED on}}$ is the measured light intensity that includes both the fluorescence and any ambient light, and $I_x^{\text{LED off}}$ is the ambient light only. Because the measurements are taken at regular time intervals (50 μs), this algorithm results in a “software lock-in” that allows the detection of relatively low fluorescence signals in the presence of relatively high background noise.

Values for the green and red emission intensities were acquired simultaneously and the resulting sums (at 32 bit each) were transferred to a PC. The computer provides power through the USB bus that is also used for bi-directional communication. A Labview-based program controls the electronics and offers the option to adjust the brightness of the LED. When a measurement is initiated, the program receives the measured green and red values and calculates their ratio. The data are saved with tags that allow identification of each measurement. A regular PC (laptop, desktop) can be used for the control or an Ultra-mobile PC (Viliv S5) for mobile measurements.

Sample holder

The sample and the dual labeled GBP are added in a miniature, 1.6 mm path length cuvette (figure 2 B). The cuvette is designed to be filled and emptied manually using a micropipette. The total volume of the cuvette is 80 μ L. The bottom was intentionally rounded to simplify solution loading and unloading. The cuvette was constructed from 3 layers of extruded polymethyl methacrylate (PMMA), cut to the desired shape either by using a laser cutter, or an in-house CNC milling center (Little machine shop.com). The layers were annealed to avoid cracking, then glued together either using thermal bonding or through solvent bonding.

Calibration

To construct the calibration curve, 200 μ L of a 6 μ M solution of dual labeled GBP was added into a quartz cuvette and the fluorescence spectrum was measured on a lab grade steady-state fluorometer (Cary Eclipse, Varian). Excitation wavelength is set at 390 nm and the spectrum was traced from 420–700 nm. 80 μ L of this solution was transferred to the custom cuvette and measured on the microfluorometer. The custom cuvette has a rounded bottom to prevent the loss of solution during manual transfer. After measurement, the solution is returned to the quartz cuvette by a micropipette with a long neck. 1 μ L of 400 μ M glucose was added to the GBP, mixed and the fluorescence spectrum was measured similarly as before. Repeating the process, 80 μ L of this mixture was transferred to the microcuvette and measured with the microfluorometer. A total of 6 consecutive additions and measurements were performed. The total volume of glucose added to the GBP solution is 6 μ L, or a 3% change in volume, which was presumed to affect the fluorescence of GBP to a negligible degree.

A second calibration curve was constructed by adding 40 μ L buffer (and subsequently 4, 8, 10 and 16 μ M glucose) to 40 μ L of the 6 μ M GBP to verify that the dual-labeled protein activity is insensitive to the 1:1 dilution (i.e. while intensity is halved, the ratio of green to red emission stays the same). Additionally, 1:1 mixing ratio is better suited for the protocol for transdermal glucose sampling as described below.

Skin sampling

An Eppendorf tube (2 ml) is cut to a height 6 mm from the top. The cylindrical portion with the collar was used to confine sampling to approximately a 1 cm² area of skin by holding it in place with a finger. 200 μ L of water was added to the well and gently aspirated with a pipette for 5 minutes. The wash was then transferred into another Eppendorf tube for storage at –20 °C or directly to assay. 40 μ L of this solution is withdrawn, mixed with 40 μ L protein solution in the custom cuvette and immediately measured using the microfluorimeter.

Results and discussion

Fluorometer design and operation

This system is intended for point-of-care measurement of low ($0.1\text{--}2\text{ }\mu\text{M}$) glucose concentrations sampled from the skin surface of patients. Therefore, the device is required to be portable, sensitive, simple to operate, rugged and safe. For these reasons, an LED and photodiodes were chosen respectively as excitation source and detector for the microfluorometer. Consequently, the overarching challenge is to make the microfluorometer sensitivity comparable to a lab-grade fluorometer that utilizes a flash lamp rather than an LED for excitation and a photomultiplier tube rather than a photodiode as detector.

The dyes used for labeling of the GBP are acrylodan ($\text{Ex}_{\text{max}} = 390\text{ nm}$) at the 255 position and ruthenium bis(2,2'-bipyridyl)-1, 10-phenanthroline-9-isothiocyanate. ($\text{Ex}_{\text{max}} = 450\text{ nm}$) at the N-terminal. The emission maxima are 530 and 610 nm, respectively. Excitation of these dyes at two different wavelengths would have been impractical as two different light sources must be employed and their light paths aligned. However, upon examination of the excitation profile of the Ru dye, it was noted that the excitation efficiency at 390 nm is ~85% of the maximum. Thus, it is possible to excite both dyes fairly well with a single light source, in this case, a violet LED. Furthermore, a fairly wide band pass filter was used to remove the “red tail” of the LED, allowing for a better excitation of the both dyes.

Two photodiodes connected to transimpedance amplifiers served as photodetectors. While lacking internal amplification as compared with avalanche photodiodes and photomultipliers, photodiodes have the advantage of not requiring high voltages for operation, are robust and mechanically stable. The price per unit is lower than other photodetectors and the overall cost is also lower because specialized high voltage power supplies are not required. Finally, because the power budget of the system is low, it can be powered directly from the USB port of a computer or a laptop.

One disadvantage of photodiodes that has to be accounted for is the lack of internal amplification which significantly decreases the signal-to-noise ratio of the detector system. The amplification has to be performed using a transimpedance amplifier to convert the photodiode's current output into voltage while simultaneously performing amplification. However, this results in the noise of the amplifier being amplified as well. Therefore, optical, electrical and algorithmic measures are implemented in order to improve signal-to-noise ratio. First, a violet LED with emission maximum that closely matched the absorption of the sensing fluorophore (acrylodan) was chosen. This is a high-brightness LED that can deliver 20 mW optical power when driven at 20 mA DC. To increase the optical output even further, the LED was driven with a square wave signal, with the peak current at 50 mA. The resulting peak power was 47 mW. Then to ensure the highest possible excitation, the excitation optical path was designed as a folded beam. (Figure 3) A mirror was positioned on the wall opposing the excitation beam entrance. In this way the excitation light passes through the cuvette at least twice and some of the returning light gets reflected from the surfaces of the excitation filter as well as from the LED back reflector. To minimize the amount of scattered excitation light directed at the photodetectors, the excitation light is positioned in a right-angle configuration. This minimizes the amount of Rayleigh scattering ideally bringing it to zero. To allow only the emission light to reach the photodetectors, band pass filters were employed that select the “red” and the “green” band. These two bands do not overlap. As the interference filters reflect the out-of-band light, each filter acts as a mirror for the other filter. In other words, the “green band” filter reflects the “red band” fluorescence, and vice versa. Because the dimensions of the cuvette cavity were practically equal to the photodiode surface, a significant percentage of the emission from the solution (> 50 %) is captured. In this way, the signal was amplified exclusively using optical means.

To further improve the signal-to noise ratio, rational design was implemented by transimpedance/secondary amplification stages. Amplifications higher than 10^8 in the first stage are not practical, requiring the use of resistors bigger than 10 MOhm. These values are approaching the surface impedance of the printed circuit board. At impedances higher than 20 MOhm, surface leakage currents are likely to start affecting the electronics operation unless special design is implemented (e.g. use of Teflon standoffs). This would have significantly increased the complexity and the cost of the system. Therefore, the bulk of the amplification (10^7) was performed in the first stage, while the second stage amplification was only 20. In this way, the amplification of the noise from the input amplifier was kept to the minimum. The first and the second stage were separated galvanically by a capacitor, effectively converting the second stage into a high-pass filter tuned to allow passage of frequencies above 1 kHz. Together with the first stage, which acted as a low-pass filter (cut-off frequency 10 kHz), the amplifiers effectively suppressed the noise on frequencies that were out of band. In order to further suppress the noise, “software lock-in” technique was implemented, as described in the experimental section.

As a result, the standard deviation of the measurement coming from the electronics noise at any light intensity was ± 1 bit of the intensity. The signal-to-noise ratio (SNR) was better than 300:1 in the both bands. These measurements were performed using high stability dye standards that were adjusted to intensities similar to that observed with the actual protein. The signal-to-noise ratio reported here is the worst SNR observed in the reference channel – the intensities in the “red” band are routinely around 300 count ± 1 when monitoring Rhodamine in ethanol. SNR in the measurement channel is higher, as the readout was slightly higher. As a result the intensity ratio can be measured with a high precision ensuring the high resolution of the whole system (see below). The ultimate limiting factor for the resolution is the quality of the protein labeling and the stability of the labels in water environment. All the protein should be adequately labeled with the both dyes; labeling only some of the molecules or over-labeling (attaching more than one label molecule per protein molecule) will affect both the calibration curve and the fluorescence intensity readout, resulting in variations of the sensor precision. Additionally, with prolonged illumination the sample start bleaching, resulting in intensity drop in both channels. This again affects the resolution and the SNR.

The visual interface of the system is shown in Figure 4. The Labview program not only visualizes the fluorescence intensity in the “red” and the “green” bands, but also displays a time course of the measurements and calculates the ratio of the two intensities. It gives an option to account for the possible offsets (i.e. due to electronics heat-up) and subtracts them from the absolute measurements. Additionally, it saves the results from measurements. Each record of measurement can be tagged with separate label for easier identification.

System testing and calibration

The first test was to run the fluorometer continuously without changing the concentration of the labeled sensing protein or glucose concentration. It was immediately obvious that the dyes bleach and the signal decreases relatively quickly (Figure 4). This is a very common phenomenon, but it is especially problematic in situations where the sample volume is low, and the intensity of the excitation is high (e.g., in fluorescence microscopy). Furthermore, we observed different bleaching rates for the two dyes. Differential bleaching would result in gradual worsening of the SNR and a calibration shift. Therefore, the number of the measurements was limited to 10 to avoid prolonged exposure of the dyes to light without sacrificing the precision.

Next, the system was calibrated according to the procedure described in the Methods section. An average of the collected 10 ratios per glucose measurement was calculated and

the result plotted in Figure 5 left. For comparison, the spectra of the test solutions were obtained on the lab grade fluorometer (Figure 5 right). As the reading of the intensity is fairly noisy due to high amplification and relatively low concentration of the sensing proteins, the peak value was calculated as average of the intensities in the range of ± 10 nm of the peak. This in effect is equivalent to opening the monochromator slits in order for the photomultiplier to sense relatively wide bands in “green: and “red”.

As can be seen, the calibration curves from the lab fluorometer and the portable system strongly resemble each other. In fact, they correlate extremely well with a correlation coefficient of 0.9855. The reasons for the difference in ratios for the lab fluorometer and the microfluorometer are several. First, no attempts were made to correct for the different spectral sensitivity in the portable fluorometer; the readout of the lab fluorometer is spectrally corrected through its software for the variations in the sensitivity of the photomultiplier tube. It should be noted that the sensitivity of the silicon photodiodes is approximately 20% higher in the “red” band as compared with the sensitivity in the “green” band. Furthermore, the two filters used have different bandwidth – 50 nm for the “green” and 60 nm for the “red”. The wider bandwidth allows more light to reach the photodetector. Finally, the filter bands are not centered around the fluorescence emission peak, as it is the case with an adjustable monochromator. All this results in output ratio closer to 1, as compared with a ratio ~ 2 for the fluorometer.

It should be also noted, that standard deviation and the associated error of the measurements performed using the portable system is smaller, despite the bleaching effects. We believe the reason for this is the enhanced light collection from the optimized optical design. The resolution of the system in the range 0–2 μM is 72 nM at 95% confidence interval. With the increase of the concentration above 2 μM , the sensitivity drops, but our approach still exhibits useful resolution (on the order of 0.3 μM) up to concentrations of ~ 5 μM .

Measurements glucose from skin samples

As a real world test, we determined the glucose on samples collected from the skin of a healthy volunteer. The results are shown in Figure 6. The blue line is the developed calibration curve of the system and is intended to serve as an “eye guide”.

First, we measured the emission ratio of a sample where 1 μL of water were added to 80 ml solution of the dual labeled GBP in PBS. We then compared this with 40 μl of GBP solution added to 40 μl deionized water. As expected, the measured ratio was identical for both solutions with a difference of less than 0.04% despite the absolute intensity dropping twice. This gives us confidence that the ratiometric approach performs well even in low-concentration measurements.

Then we measured 3 “real” samples from our volunteers collected as described in Methods. Sample +1 was from the skin of a healthy volunteer who was fasting for several hours (before lunch). The detected concentration in the wash was ~ 1.3 μM . Sample +2 was a sample from the same person approximately one hour after lunch. As expected, increased glucose concentration was found in the wash – approximately 1.7 μM . Finally, the wash from the skin of a healthy volunteer who consumed two chocolate bars after lunch was also measured. Even higher glucose concentration ~ 1.8 μM – was found in that wash.

Conclusion

Presented results suggest that the microfluorometer system is a promising tool for accurate measurements of low glucose concentrations (< 10 μM) in biological samples. The limit of detection was ~ 0.08 μM . It compares favorably with a number of other recently published

systems that target measurements of low glucose concentrations. The deviation of the measured ratios is within the measurement uncertainty. In future, we will focus on methods for decreasing the photo bleaching so that a better estimate of the actual glucose concentration becomes possible. Further testing on a larger cohort of patients is also underway with comparisons with blood glucose levels.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
The glucose binding protein (GBP) undergoes conformational change in the presence of glucose.

Figure 2. Ratiometric fluorometer. Top) Overall view of the system with the top cover removed. Fluorometer is attached to ultra-mobile PC ready for point-of-care measurements. Middle) Side view showing the position of the LED, photodiodes and the cuvette. Bottom left) Plastic cuvette with 1.6 mm emission pathlength; 100 μ L pipette tip shows filling of the cuvette; Bottom right) Fluorometer with the cuvette placing.

Figure 3.

Block schematics of the ratiometric fluorometer. VCCS – voltage controlled current source; ExF – excitation filter. PDR – photodiode measuring the “red” band; PDG – photodiode measuring the “green” band; ADC - analog-to-digital converter; μ P – microprocessor; USB – communication to universal serial bus.

Figure 4. Labview-based Visual interface for micro fluorometer control. It allows for accessing the ratio of the two intensities, as well as raw intensities from the channels. Offset subtraction can be also introduced. Data can be saved with a tag.

Figure 5.

Calibration of the portable system as compared with laboratory fluorometer. Left panel: comparison of the calibration curves obtained with the system and the fluorometer. Note the significantly lower standard deviations of the portable system. Right panel: Spectra of the GBP solution at glucose concentrations corresponding to the calibration curve.

Figure 6. Data showing the real world capability of the portable fluorometer. Standard glucose solutions (Blue diamonds) and transdermal samples from an adult and a blank (Green diamonds).