A “plasmonic cuvette”: dye chemistry coupled to plasmonic interferometry for glucose sensing

Abstract: A non-invasive method for the detection of glucose is sought by millions of diabetic patients to improve personal management of blood glucose over a lifetime. In this work, the synergistic advantage of combining plasmonic interferometry with an enzyme-driven dye assay yields an optical sensor capable of detecting glucose in saliva with high sensitivity and selectivity. The sensor, coined a “plasmonic cuvette,” is built around a nano-scale groove-slit-groove (GSG) plasmonic interferometer coupled to an Amplex–red/Glucose–oxidase/Glucose (AR/GOx/Glucose) assay. The proposed device is highly sensitive, with a measured intensity change of $1.7 \times 10^5 \% / \text{M}$ (i.e., one order of magnitude more sensitive than without assay) and highly specific for glucose sensing in picoliter volumes, across the physiological range of glucose concentrations found in human saliva (20–240 μM). Real-time glucose monitoring in saliva is achieved by performing a detailed study of the underlying enzyme-driven reactions to determine and tune the effective rate constants in order to reduce the overall assay reaction time to ~2 min. The results reported suggest that by opportunely choosing the appropriate dye chemistry, a plasmonic cuvette can be turned into a general, real-time sensing scheme for detection of any molecular target, with high sensitivity and selectivity, within extremely low volumes of biological fluid (down to femtoliters). Hereby, we present the results on glucose detection in artificial saliva as a notable and clinically relevant case study.

Keywords: Plasmonics; plasmonic interferometry; Amplex red; kinetics; glucose sensing; diabetes.

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1 Introduction

Diabetes mellitus is a chronic metabolic disorder that affects over 347 million people worldwide [1]. Although there are many causes for diabetes, the primary symptom of this disease is excessive glucose levels in the blood. The long-term complications associated with diabetes include cardiovascular disease, chronic renal failure, and diabetic retinopathy, all of which can be reduced significantly by controlling blood glucose levels using a blood glucose meter (BGM). Traditional BGMs employ bio-electrochemical schemes that use glucose oxidase (GOx) or glucose dehydrogenase (GDH) immobilized on the surface of a disposable electrode [2]. These monitoring schemes are non-toxic and have high selectivity; however, the detection limit of commercial BGMs is ~2.8 mM (50 mg/dL) [3], which prevents their use in fluids such as tears or saliva. These fluids, while not requiring invasive extraction are characterized by glucose concentrations that are typically two orders of magnitude lower than what is found in blood.

Over the past decade, extensive research has focused on optical, rather than electrochemical methods, with the goal of reducing the limit of detection and achieving non-invasive sensing [4–12]. Optical non-invasive detection schemes assume a direct correlation between the glucose levels found in blood and other bodily fluids; this correlation is well established for interstitial fluids [13] and saliva [14, 15]. Saliva is a particularly attractive candidate for non-invasive glucose sensing since it is almost always present and easy to acquire (unlike tears). These optical biosensors can be further improved by employing...
metal nanoparticles or planar metal films with nano-corrugations in order to favorably manipulate the incident light beam [16–21]. Example techniques include surface plasmon resonance (SPR) [22, 23], grating couplers [24], and plasmonic interferometers [25, 26]. In all these techniques, incident light is coupled to surface plasmon polaritons (SPPs) that are electromagnetic waves supported by electron density fluctuations in noble metals (e.g., silver and gold) [27]. SPPs are generated from localized sources (e.g., grooves) and propagate away from the source and along the metal/dielectric interface to interfere with each other and produce local field intensity minima and maxima. Since SPPs are confined at the metal surface, they are highly sensitive to changes in the refractive index of the dielectric material, a property useful in developing ultra-sensitive, compact and real-time biosensors.

Herein we demonstrate a sensing scheme denominated “plasmonic cuvette” (Figure 1) obtained by coupling the spectroscopic fingerprinting capabilities of a groove-slit-groove (GSG) plasmonic interferometer (Figure 1G) to an Amplex–red/Glucose–oxidase/Glucose (AR/GOx/Glucose) enzyme-driven dye assay (Figure 1A–C) that adds selectivity for glucose detection, and further enhances the device sensitivity by one order of magnitude. The resulting sensor offers real-time sensitivity toward glucose in extremely small sensing volumes (i.e., ≤12 pL), and exhibits glucose selectivity in complex mixtures such as a 50 mM sodium phosphate buffer solution and “artificial”

![Figure 1](image_url)

**Figure 1** Plasmonic cuvette: plasmonic interferometry coupled to dye chemistry. (A) The Amplex–red/Glucose–oxidase/Glucose (AR/GOx/Glucose) assay consumes glucose and produces resorufin, a red-fluorescent molecule, in a 1:1 stoichiometric ratio via enzymatic reactions; measurement of steady-state resorufin concentration leads to determination of glucose concentration initially present in solution; (B) Reaction 1: oxidation of β-D-glucose to D-gluconolactone by O2 to produce H2O2, catalyzed by GOx; (C) Reaction 2: oxidation of Amplex red (colorless) into resorufin (red), catalyzed by HRP; (D) Reaction 3: side reaction with low yield that further oxidizes resorufin to an optically inactive product; (E) the effective rate constants for the three reactions (K1, K2, and K3) were determined by time-dependent kinetic studies of resorufin absorption; (F) absorption cross-section (σ) and extinction coefficient (ε) of resorufin as a function of wavelength, needed to extract resorufin (and therefore glucose) concentration from absorption measurements; (G) schematic of a plasmonic interferometer; (H) sample data from the plasmonic cuvette: spectral absorption is correlated with resorufin concentration.
saliva, over the physiological range of glucose concentration in saliva (20–240 μM) [28]. These results demonstrate the viability of measuring the concentration of glucose in saliva, which is a complex mixture of proteins, salts, and urea [29]. More details about the experimental methods can be found in Appendix A.

The plasmonic cuvette (shown in Figure 1G) offers several advantages over a traditional UV–Visible (UV–Vis) cuvette (Figure 1E). First, the sensed volume in a plasmonic cuvette is 12 pL (compared to ≥240 μL for the UV–Vis cuvette), thus reducing the amount of sample and reagents required by seven orders of magnitude. Second, the plasmonic cuvette can be integrated with a microfluidic channel to achieve more uniform mixing than what is normally possible in a UV–Vis cuvette. Third, the plasmonic cuvette has a very short effective path length (<20 μm) compared to a UV-Vis cuvette (~1 cm). The shorter path length of a plasmonic cuvette allows the response of the system to remain linear over a wider range of analyte concentrations and avoids the necessity to either dilute the solution or reduce the path length of the UV-Vis cuvette at higher analyte concentrations in order to have a measurable transmittance.

Figure 1A provides an overview of the assay reactions that, starting from one molecule of glucose, end up generating one molecule of optically active resorufin, which can be identified and quantified more easily using optical methods, such as plasmonic interferometry. Typically, the fluorescence intensity from resorufin is used to quantify the initial glucose concentration. However, the emission efficiency strongly depends on the pH of the solution, as well as on the chemical components dissolved in solution, thus affecting the quantum yield of the emitter, and making it difficult to directly correlate the measured fluorescence intensity with the initial glucose concentration. Here, we propose the use of the absorption (rather than emission) properties of resorufin to reliably determine the glucose concentration. We characterize the absorption cross section of the emitter in solution, and calibrate the assay for accurate glucose detection. Plasmonic interferometers are used to amplify and monitor the absorption changes in solution as well as quantify the glucose concentration in very small sample volumes, with high sensitivity and selectivity.

Details of each enzymatic reaction occurring in the assay are shown in Figure 1B–D. Reaction 1 (Figure 1B) describes the oxidation of β-D-glucose to D-glucosone by oxygen catalyzed by GOx to yield H₂O₂ as a product [30]. The H₂O₂ generated in Reaction 1 is utilized by horseradish peroxidase (HRP) to oxidize Amplex red (AR), a colorless, non-fluorescent compound, to resorufin, a red, fluorescent compound (Reaction 2, Figure 1C) [31–33]. Reaction 3 (Figure 1D) is a much slower, H₂O₂-limited side reaction that further oxidizes resorufin into an optically inactive product (OIP), thought to be a complex polymer [30, 33, 34]. Reactions 1 and 2 are commonly used to detect glucose in a wide variety of applications, such as determining glucose consumption by cancerous cells to better understand tumorigenesis [35], or quantifying starch and glucose concentrations in food [36]. In these applications, the fluorescence properties of resorufin are preferred because of the high signal-to-noise ratio (Figure 1E). By contrast, this work employs the absorption properties of resorufin (Figure 1F) in order to determine a quantifiable change in the optical transmission response of the plasmonic cuvette, which can be correlated to the glucose concentration initially present in solution. The foundation of the plasmonic cuvette, a groove-slit-groove (GSG) plasmonic interferometer, is shown in Figure 1G [26]. When light of wavelength λ is incident upon a groove, an SPP wave is generated and propagates through the sample solution toward the center slit, which is a distance p away from the groove (p is also known as the arm length of the interferometer). The counter propagating SPP waves originating from the two grooves interfere constructively or destructively at the slit location depending on p, λ, and the refractive index n of the dielectric resting on top of the metal. The output transmission spectrum will be a convolution of the incident light and the net SPP fields at the slit location. Changes in the intensity or shifts in the wavelength of the output spectrum provide information about the concentration and types of analytes present (Figure 1H).

We also report a detailed study of the enzyme kinetics involved in the AR/GOx/Glucose assay: the effective rate constants are found and then used to tune the reaction time and range of applicability of the assay to cover the physiological range of glucose in saliva, in real-time.

2 Results and discussion

2.1 Enhancing the sensitivity of plasmonic interferometers

Both the plasmonic and UV-Vis cuvettes rely on the same figure of merit to quantify sensitivity, which itself depends on the ability of the solution to absorb incident light. The relevant figure of merit is the relative intensity change (ΔI/I₀), defined as

$$\frac{\Delta I}{I_0} = \frac{I_{\text{glucose}} - I_0}{I_0}$$

(1)
where $I_{\text{glucose}}$ is the light intensity transmitted through the slit of the plasmonic interferometer at a non-zero glucose concentration, while $I_0$ corresponds to the absence of glucose. The light intensity transmitted through the slit of a GSG interferometer, normalized to the light intensity transmitted through a single slit (SS) is [26]:

$$ I_{T,GSG}/I_{SS} = 1 + \beta e^{i (k_{SPP} + k_\theta) \sin \theta} + e^{i (k_{SPP} - k_\theta) \sin \theta} $$

(2)

where $p_1$ and $p_2$ are the lengths of the left and right arms of the GSG plasmonic interferometer, respectively, $\lambda$ is the free space incident wavelength, $k_{SPP} = 2\pi n_{SPP}/\lambda$ and $k = 2\pi n/\lambda$, where $n_{SPP}$ is the complex refractive index of the SPP given by $n_{SPP} = \sqrt{\varepsilon_m \varepsilon_d} / (\varepsilon_m + \varepsilon_d)$ [27] with $\varepsilon_m$ as the complex dielectric constant of the metal and $\varepsilon_d$ as the complex dielectric constant of the material above the metal; $n$ is the refractive index of the dielectric material SPPs propagate through; $\beta$ and $\phi_g$ are the effective excitation efficiency and scattering phase of an SPP; angle of incidence $\theta$ is set to 0. As a result of the interference process between the SPP waves and the incident light beam, the light intensity transmitted through the slit can be either enhanced or suppressed, depending on whether constructive or destructive interference occurs.

Figure 2A shows the measured relative intensity change defined in Eq. (1) as a function of incident wavelength, when the assay is present (red solid line) or absent (blue dashed line); the initial glucose concentration is $[G]_{t=0} = 250 \pm 6 \mu M$ in both cases. The AR/GOx/Glucose assay produces 1 mol of resorufin for every mol of glucose consumed. Consequently, any change in light intensity corresponds directly to a change in glucose concentration – this figure clearly shows that the assay is effective in increasing the sensitivity of the device. In this device, two distinct optical processes are occurring: direct absorption of the incident light (characterized by the absorption cross-section curve in Figure 1F) and absorption of the groove-generated surface plasmon polaritons (SPPs). In this regard, the spectrum can be divided into three distinct regions. At shorter wavelengths ($450 \text{ nm} < \lambda < 510 \text{ nm}$), the direct absorption of light by resorufin is low enough that the two processes can be resolved. The pronounced peaks in this region of the spectrum can be attributed to interference between counter-propagating SPP waves; the shift in peak position as well as the intensity change can be correlated to a refractive index change, as initially reported in Ref. [26]. At wavelengths between 510 nm and 590 nm, resorufin is strongly absorbing; thus, bulk absorption dominates over plasmonic interference and absorption. The presence of this absorption band, which closely resembles the shape of the absorption spectrum of resorufin, ensures the device is selective towards glucose and can be attributed to the high specificity of GOx to glucose in the assay. At $\lambda_{\text{max}} = 571 \text{ nm}$, the absolute value of the relative intensity change is at a maximum and goes from 2.8% (without assay) to 41.7% (with assay). At wavelengths above 590 nm, resorufin does not absorb light significantly, so any relative intensity change is due solely to changes in the SPPs propagation constant and interference behavior, which depends on the refractive index of the solution. This observation suggests that differences in

Figure 2 Enhancing the sensitivity of a plasmonic interferometer with the dye assay. (A) Relative intensity change plotted as a function of wavelength for a groove-slit-groove (GSG) plasmonic interferometer with 250±6 μM glucose in the presence (red solid line) or absence (blue dashed line) of the assay. Both spectra are normalized to a reaction mixture with 0 μM glucose (black solid line). (B) Calibration curves for the same GSG device used in part (A) as function of glucose concentration in the presence (red squares, $\lambda = 571 \text{ nm}$) or absence (blue circles, $\lambda = 628 \text{ nm}$) of the assay.
the two spectra are a result of the enzymes and resorufin altering the refractive index of the solution (both the real and imaginary parts). In theory, any wavelength in this spectrum could be used for detection, but the best sensitivity is achieved at $\lambda_{\text{max}} = 571$ nm. Note that Figure 1H displays the same data, “with assay”, but over a range of glucose concentrations between 0 and 250 $\mu$m, demonstrating the expanded range of applicability provided by the coupling of dye chemistry with plasmonic interferometry.

Figure 2B shows the relative intensity change as a function of initial glucose concentration with and without the dye assay present in the microfluidic channel. When the assay is absent (blue circles), the calibration curve yields a sensitivity of 0.2×10^6%/M. When the assay is present (red squares), a linear fit of the data yields a sensitivity of 1.7×10^6%/M, which corresponds to an 8.5× increase (N.B.: the sensitivity is defined as the absolute value of the slope of the linear fit). The measurements were performed on the same plasmonic interferometer, but the detection wavelengths were chosen as $\lambda = 571$ nm (with assay) and $\lambda = 628$ nm (without assay) to maximize the sensitivity of the device in each case. For comparison, the sensitivity of our previously reported GSG plasmonic interferometer – with $p_r = 5.70$ $\mu$m and $p_p = 9.75$ $\mu$m, $\lambda = 590$ nm, no assay present – yielded a sensitivity of 0.4×10^6%/M [26]. The data presented here demonstrates that not only can the assay be used with different plasmonic interferometers (i.e., different arm lengths, $p$) to detect glucose, but that the sensitivity and selectivity of the device is significantly enhanced by the presence of the assay.

### 2.2 Development of an optical ruler for resorufin

Since the absorption properties of resorufin affect the output signal of the plasmonic interferometer, an optical “ruler” is first determined based on the optical properties of resorufin and the buffer composition in order to optimize the performance of the plasmonic cuvette. In order to exploit the 1:1 stoichiometry of the dye assay, the optical parameters are measured at steady-state concentration of resorufin by optical means to retrieve the initial glucose concentration in solution.

One important parameter is the absorption cross-section ($\sigma$) of resorufin, in units of cm$^2$, defined as the effective cross-sectional area of a resorufin molecule when light is incident upon it. The absorption cross-section can be defined at all wavelengths and it can slightly depend on the nature and composition of the buffer solution. Knowledge of $\sigma$ is required to determine the absolute concentration of resorufin, and therefore glucose initially present in solution. Related to $\sigma$ is the extinction coefficient ($\varepsilon$) of resorufin, in units of M$^{-1}$ cm$^{-1}$, which is a measure of how strongly the optically-active molecules absorb incident light. Shown in Figure 1F is a plot of $\sigma$ as a function of wavelength on the left vertical axis. The extinction coefficient ($\varepsilon$) of resorufin is plotted on the right axis and is related to $\sigma$ by

$$\sigma = \frac{c \log_{10}(10)}{\rho} \varepsilon = (3.824 \times 10^{-21} \text{ mol}) \varepsilon$$

where $c$ and $\rho$ are the molar concentration and the density of resorufin, respectively (see Appendix B, for a full derivation, and Appendix C for the experimental determination of $\sigma$ from absorption measurements in solution). The incident wavelength chosen for this work was $\lambda_{\text{max}} = 571$ nm where $\sigma_{571}$ = (2.05±0.08)×10^-16 cm$^2$ and $\varepsilon_{571}$ = (5.4±0.2)×10^4 M$^{-1}$ cm$^{-1}$ (compared to the literature value of $\varepsilon_{571}$ = (5.4±0.4)×10^4 M$^{-1}$ cm$^{-1}$) [31, 37]. The accuracy of the $\varepsilon_{571}$ value reported here confirms the validity of this method to obtain $\sigma$ and $\varepsilon$ at other wavelengths. Figure 1F expands the range of applicable wavelengths for resorufin detection to more than just its peak absorption wavelength. This method can be generalized for deriving the absorption properties for any optically active molecule – once $\sigma$ or $\varepsilon$ has been found, the optimal wavelength can be selected to use with the sensor. Additionally, because every molecule has its own unique $\sigma$ and $\varepsilon$ wavelength dependence, the plasmonic cuvette is capable of multiplex sensing, i.e., two or more optically active biochemical analytes that are present on the chip could be identified and quantified simultaneously.

### 2.3 Mathematical model of AR/GOx/Glucose assay to determine the reaction constants $K_1$, $K_2$, and $K_3$

The AR/GOx/Glucose assay typically requires an incubation time of >30 min for the reactions to proceed to completion [37]. The incubation time needs to be shortened significantly if the plasmonic cuvette is to be used as a real-time sensor for glucose. Efforts have been made to characterize the factors that affect the incubation time of the coupled GOx- and HRP-dependent reactions [38]. A study by Nakajima et al. reduced the incubation time to 4.8 s by photochemically arranging the HRP and GOx molecules in a specific pattern inside a microfluidic PDMS channel [39].

Because all of the reagents are simultaneously present in solution in a plasmonic cuvette sample, the best approach is to increase the rates of the three reactions (see Figure 1B–D, corresponding to rate constants $K_1$, $K_2$, and $K_3$.
and $K_3$, respectively). The values for these rate constants can be obtained by fitting the change in the concentration of resorufin as the reaction progresses. Using first-order kinetics, a model for the time-dependent concentrations can be established with the following rate equations:

$$\frac{d[G]}{dt} = -K_1[G][O_2]$$  \hspace{1cm} (4A)
$$\frac{d[O_2]}{dt} = 0$$  \hspace{1cm} (4B)
$$\frac{d[H_2O_2]}{dt} = K_1[G][O_2] - K_2[H_2O_2][AR] - K_3[H_2O_2][R]$$  \hspace{1cm} (4C)
$$\frac{d[AR]}{dt} = -K_2[H_2O_2][AR]$$  \hspace{1cm} (4D)
$$\frac{d[R]}{dt} = K_2[H_2O_2][AR] - K_3[H_2O_2][R]$$  \hspace{1cm} (4E)
$$\frac{d[OIP]}{dt} = K_3[H_2O_2][R]$$  \hspace{1cm} (4F)

where $G$ is glucose, AR is Amplex red, R is resorufin, and OIP is the optically inactive product generated in Reaction 3. This non-linear system of differential equations was solved numerically to fit the experimental data, with the initial concentrations of the six reagents as initial parameters and the rate constants as fitting parameters. The concentration of resorufin at time $t$, $[R]_t$, was found by measuring the transmittance ($T$) as a function of time $t$, and using the following formula (see Appendix B for a derivation):

$$[R]_t = \frac{-\log_{10}(T)}{\varepsilon L}$$  \hspace{1cm} (5)

where $\varepsilon$ is the extinction coefficient of resorufin and $L$ is the path length of the cuvette.

### 2.4 Determination of $K_1$

To obtain the experimental concentration of resorufin as a function of time, the transmittance recorder at each time $t$ (Figure 3A) was monitored for reaction mixtures in a 50 mM sodium phosphate buffer (pH 7.4) with 280±6 μM AR, 5.5±0.1 nM HRP and 82.5±0.7 nM GOx, and then converted into resorufin concentration vs. time using Eq. (5), as reported in Figure 3B. The concentration of glucose was varied between 0 and 210 μM. The initial concentration of Amplex red was set to 280 μM for two reasons: (i) to ensure a 1:1 stoichiometric ratio between AR and glucose while allowing the initial concentration of glucose to vary across the entire physiological range of glucose in human saliva, (ii) to prevent precipitation of AR (i.e., the solubility limit of AR is 300 μM in the aqueous buffer solutions used here) [31].

Although it is possible to solve for $K_1$, $K_2$, and $K_3$, respectively. The values for these rate constants can be obtained by fitting the change in the concentration of resorufin as the reaction progresses. Using first-order kinetics, a model for the time-dependent concentrations can be established with the following rate equations:

$$\frac{d[G]}{dt} = -K_1[G][O_2]$$  \hspace{1cm} (4A)
$$\frac{d[O_2]}{dt} = 0$$  \hspace{1cm} (4B)
$$\frac{d[H_2O_2]}{dt} = K_1[G][O_2] - K_2[H_2O_2][AR] - K_3[H_2O_2][R]$$  \hspace{1cm} (4C)
$$\frac{d[AR]}{dt} = -K_2[H_2O_2][AR]$$  \hspace{1cm} (4D)
$$\frac{d[R]}{dt} = K_2[H_2O_2][AR] - K_3[H_2O_2][R]$$  \hspace{1cm} (4E)
$$\frac{d[OIP]}{dt} = K_3[H_2O_2][R]$$  \hspace{1cm} (4F)

where $G$ is glucose, AR is Amplex red, R is resorufin, and OIP is the optically inactive product generated in Reaction 3. This non-linear system of differential equations was solved numerically to fit the experimental data, with the initial concentrations of the six reagents as initial parameters and the rate constants as fitting parameters. The concentration of resorufin at time $t$, $[R]_t$, was found by measuring the transmittance ($T$) as a function of time $t$, and using the following formula (see Appendix B for a derivation):

$$[R]_t = \frac{-\log_{10}(T)}{\varepsilon L}$$  \hspace{1cm} (5)

where $\varepsilon$ is the extinction coefficient of resorufin and $L$ is the path length of the cuvette.

Figure 3 Determination of $K_1$. (A) Intensity of light transmitted through the assay mixture plotted as a function of time at different concentrations of glucose. The transmittance through a cuvette ($L=0.2$ cm) is monitored every 1 s for 2000 s at $\lambda_{max}=571$ nm; the data points are plotted in steps of 50 s for clarity (the confidence band interval is kept at 1 s). Each measurement is performed three times; the data show the average of the three trials. (B) Concentration of resorufin plotted as a function of time using Eq. (5) (symbols). A non-linear first order kinetic model was used to fit the data (solid lines).
by the fact that Reaction 3 is slower than Reaction 2 ($K_3 < K_2$) and that Reaction 3 will occur only after there is a buildup of $\text{H}_2\text{O}_2$ in the reaction mixture. However, $\text{H}_2\text{O}_2$ is readily used up by Reaction 2, Eqs. (4C) and (4D), so that it does not contribute significantly to Reaction 3, i.e., Eqs. (4E) and (4F). Zhou et al. demonstrated that a decrease in resorufin concentration (i.e., the initiation of Reaction 3 to generate OIP) is observed only when the ratio of solution concentrations of $\text{H}_2\text{O}_2$ to AR is 2:1 [31]. In the full assay, the initial concentrations of resorufin and $\text{H}_2\text{O}_2$ always start at zero. Using the model to simulate the concentration of all six reagents as a function of time, the concentration of $\text{H}_2\text{O}_2$ never exceeds $10 \mu \text{M}$ (see Appendix G, Figure 10) when the highest initial concentration of glucose (210 $\mu \text{M}$) was used. This concentration of $\text{H}_2\text{O}_2$ is significantly lower than that of AR in solution.

To confirm that resorufin is the only optically active molecule in the buffer solution, the spectral absorption (defined as $A=1-T$ in the case of negligible reflection) was measured at different times and normalized to the absorption curve after the system reached saturation. The absorption spectrum does not change over the course of the experiment, thus confirming that other optically active products were not generated. This observation gives further credence to setting $K_i = 0$ when solving for $K_i$. If $K_i$ was not zero, then resorufin would undergo further oxidation to OIP, which would change the absorption profile of the reaction over time, which is not observed (see Appendix H, Figure 11).

Shown in Figure 3A are transmittance curves plotted as a function of time for several different initial concentrations of glucose. Figure 3B plots the converted transmittance data to resorufin concentration over time using Eq. (5). Although $[\text{G}]_{0,0}$ was known a priori, this quantity was used as a variable parameter in the fitting code to test the ability of our plasmonic cuvette as an effective sensing scheme to retrieve the glucose concentration initially present in solution; the numbers reported in Figure 3B refer to the fits (reported as solid lines). In all cases, the fitted value matches the concentration of resorufin at saturation, confirming the accuracy of the model. For each value of $[\text{G}]_{0,0}$, a fitted value for $K_i$ and $[\text{G}]_{0,0}$ was found. The weighted average of all $K_i$ data yields $K_i = 7.3 \pm 0.3$ $\text{M}^{-1} \text{s}^{-1}$ (see Appendix F, Figure 9).

The fitted curves show an excellent correlation to the experimental data, with a small discrepancy occurring when $[\text{G}]_{0,0} > 114 \mu \text{M}$ at long reaction times. There are two possible explanations for this slight discrepancy between the model and experiment. First, there may be local regions of depleted oxygen, which can be prevented by bubbling oxygen into the buffer solution prior to experimentation. Second, Amplex red may be photo-oxidized to resorufin [40–42]. In order to test this effect, three neutral density filters with different attenuation factors were used to reduce incident intensity. However, no significant change was observed in the measured concentration of resorufin (see Appendix I, Figure 12), thus eliminating this possibility.

### 2.5 Tuning the reaction time of the AR/GOx/Glucose assay

When applying the plasmonic cuvette to real-time glucose sensing, there are three time-dependent steps that must be considered: (i) the reaction time of the AR/GOx/Glucose assay (typically between 30 and 50 min for standard assays); (ii) the flow time required to deliver the reacted solution to the surface of the sensor (8–10 s); and (iii) the data acquisition time (∼60 s). Since the reaction time of the assay is the longest, this property was investigated further and reduced by increasing the GOx concentration of Reaction 1; this reaction was found to have the smallest rate constant and therefore, was recognized as the rate-limiting step in the assay (Table 1).

Figure 4A reports the concentration of resorufin measured as a function of time for various concentrations of GOx; each curve is normalized by its own saturation value. As expected, increasing [GOx] makes the reaction run faster, but the effect saturates as [GOx] increases. To quantify this effect, Figure 4B plots the reaction time ($\tau$, defined as the time it takes for $[\text{R}]_i$ to reach 63.2% of its saturation value) as a function of [GOx]. For example, increasing [GOx] by a factor of 10 (from 82.5 ± 0.7 $\mu \text{M}$ to 825 ± 7 $\mu \text{M}$) shortens the reaction time from 583.5 s to 136.5 s, i.e., a factor of 4.3×. A non-linear dependence between $K_i$ and [GOx] is observed when the data from each concentration of GOx was fitted for a new $K_i$ value, with $K_2$ and $K_3$ unchanged. The non-linear behavior may be caused by aggregation of enzyme at higher concentrations, thus reducing the amount of active sites available to react with glucose (see Appendix J, Figure 13A). At the other extreme, when Reaction 1 occurs very quickly, Reaction 2 takes over as the rate-limiting step in the overall array. A similar experiment was

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**Table 1** Rate constants for the reactions in the AR/GOx/Glucose assay.

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<thead>
<tr>
<th>Rate constant</th>
<th>Enzyme concentrations</th>
<th>Mean value (m$^{-1}$ s$^{-1}$)</th>
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<tbody>
<tr>
<td>$K_1$</td>
<td>HRP: 5.5 nM; GOx: 82.5 nM</td>
<td>7.3 ± 0.3</td>
</tr>
<tr>
<td>$K_2$</td>
<td>HRP: 5.5 nM</td>
<td>135 ± 5</td>
</tr>
<tr>
<td>$K_3$</td>
<td>HRP: 5.5 nM</td>
<td>56 ± 7</td>
</tr>
</tbody>
</table>

Data taken at 25°C in 50 mM sodium phosphate buffer (pH 7.4), $[\text{AR}]_{0}=280\pm6 \mu \text{M}$, $[\text{HRP}]=5.5\pm0.1 \text{ nM}$, $[\text{GOx}]=82.5\pm0.7 \text{ nM}$, and $[\text{G}]_{0,0}=0–200 \mu \text{M}$.
performed for Reaction 3 only. The value for \( K_3 \) was found as a function of [HRP] by fitting the experimental data to Eqs. (4E) and (4F) with [AR]=0 at all times. The relationship between \( K_3 \) and the concentration of HRP was found to be linear, as expected from Eq. (4E), with a slope of \( K'_3 = (1.45 \pm 0.04) \times 10^{10} \text{ m}^{-2} \text{ s}^{-1} \) (see Appendix J, Figure 13B).

### 2.6 Selectivity test of glucose in artificial saliva

Although the plasmonic cuvette demonstrated high selectivity for glucose in a 50 mM sodium phosphate buffer solution, clinical samples of saliva are more complex and include a mixture of glycoproteins, enzymes, urea and electrolytes [29]. The following experiments were performed in modified Fusayama artificial saliva (MFAS), which differs from actual saliva by the absence of large (>12 kDa) proteins and enzymes (e.g., immunoglobulin and \( \alpha \)-Amylase, respectively). MFAS contains 16.7 mM of urea; in contrast, the concentration of urea in actual saliva is typically 2–3 mM. Figure 5 shows the production of resorufin over time using the AR/GOx/Glucose assay in MFAS to demonstrate the selectivity of glucose in a complex mixture and in 50 mM sodium phosphate buffer solution as a control. The black squares correspond to the experiment in MFAS (diluted 7× to increase the solubility of the urea and salts in the mixture) without glucose; as expected, in the absence of glucose molecules, the reaction to produce resorufin does not occur. When glucose is included in the reaction mixture, the assay proceeds normally (red circles). Since the composition of MFAS is different than that of 50 mM sodium phosphate buffer solution, the value of \( \varepsilon_{571} \) for MFAS was found to be \((4.6 \pm 0.2) \times 10^4 \text{ m}^{-1} \text{ cm}^{-1}\). To quantify the difference between MFAS and the buffer solution, the experiment was repeated with the same \( [G]_{i,w} \) value in buffer solution (blue triangles). The two curves are quite similar: at the saturation time (t=1855 s) the difference between the two curves is ~74%. Nothing in the MFAS solution should react with resorufin; thus, the discrepancy between the two curves can be attributed to a change in the overall kinetics of the assay, causing resorufin to be generated at a slightly faster rate in MFAS. A kinetic analysis of the AR/GOx/Glucose...

![Figure 4](image-url) Relationship between assay time and GOx concentration for Reaction 1. (A) Normalized concentration of resorufin plotted as functions of time at various GOx concentrations. The black arrow indicates the direction of decreasing reaction time. (B) Reaction time, \( \tau \) is plotted as a function of GOx concentration.

![Figure 5](image-url) Effects of artificial saliva on the AR/GOx/Glucose assay. The concentration of resorufin plotted as a function of time for reaction mixtures initiated with \( [\text{Glucose}]_{i,w} = 13 \pm 0.6 \mu\text{M} \) in either (1) artificial saliva diluted in 50 mM sodium phosphate buffer (1:6 v/v ratio, red circles) or (2) 50 mM sodium phosphate buffer (blue triangles). The control experiment is diluted artificial saliva with 0 \( \mu\text{M} \) glucose (black squares).
assay in complex mixtures such as human saliva at different pH and temperatures will be reported elsewhere.

3 Conclusion

In conclusion, this work introduces the concept of a plasmonic cuvette: a device that couples an enzyme-driven dye assay, specifically tailored for glucose sensing, to plasmonic interferometry to produce a highly sensitive, selective, real-time, compact optical glucose meter. The sensitivity was found to be 1.7×10^10%/m within the physiological range of glucose in saliva (20–240 μM). This high sensitivity can be attributed to the strongly absorbing properties of resorufin, which is characterized by unique spectral fingerprints. Our plasmonic cuvette is able to detect glucose concentrations with a resolution (limit of detection, LOD) of ~0.1 μM. By varying the arm length of the plasmonic interferometer as well as the enzyme concentrations in the dye assay, the plasmonic cuvette studied in this report has been specifically optimized for detection of glucose concentrations in the 20–240 μM range, typically found in human saliva. Our method enables detection limits that are four orders of magnitude smaller than what is currently achievable using conventional electrochemical blood glucose meters (LOD=2.8 mm), employing ~100 times lower sample volumes. The assay time (~2 min) was reduced by altering the enzyme concentration of the rate-limiting reaction (i.e., Reaction 1), after determining specific reaction rate constants that underlie the assay and fitting a first-order non-linear kinetic model to the experimental data. Finally, the plasmonic cuvette was tested with artificial saliva to demonstrate the selective detection of glucose in a complex mixture of urea and salts typically found in human saliva. This report demonstrates the first step towards a sensitive and selective sensor for glucose in saliva. However, the methodology reported here is just a proof of concept of a more general approach involving plasmonic interferometry coupled to other dye assays and sensing schemes to screen for various analytes in bodily fluids such as serum, saliva and tears.

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Appendix A: Experimental methods

Reagents

10-Acetyl-3,7-dihydroxyphenoxazine (Amplex red, or AR) and 7-Hydroxy-3H-phenoxazin-3-one sodium salt (resorufin sodium salt) were purchased from Invitrogen (Carlsbad, CA). A stock solution of 19.5 mM AR and 4.5 mM resorufin sodium salt was prepared in anhydrous dimethylsulfoxide (DMSO), aliquoted and stored at -20°C. Horseradish peroxidase (HRP) from Armoracia rusticana (253 purpuragolin U/mg, P-8375, ECl.11.1.7), glucose oxidase (GOx) from Aspergillus niger (100 kU/mg, G7141, ECl.1.3.4), D-(+)-Glucose and urea were purchased from Sigma (St. Louis, MO). Stock concentrations of HRP (10 U/mL) and GOx (1 U/mL) were determined spectrophotometrically using molar extinction coefficients ε_{280nm}=102,000 M^{-1} cm^{-1} [43], and ε_{290nm}=267,200 M^{-1} cm^{-1} [44], respectively. A stock solution of 0.5 mM D-(+)-Glucose was prepared and subsequently allowed to stand for 1 h to complete the mutarotation reaction. The concentration of hydrogen peroxide (35% w/w H₂O₂ from Acros Organics) was determined spectrophotometrically at λ=240 nm using a molar extinction coefficient of ε_{290nm}=43.6 M^{-1} cm^{-1} [45]. Initial reaction mixtures of the AR/GOx/Glucose assay were prepared at ambient temperature and consisted of 280±6 μM AR, 5.5±0.1 nm HRP and 82.5±0.7 nm GOx dissolved in a 50 mM sodium phosphate buffer solution (pH 7.4) unless otherwise stated. The enzyme stock solutions were thawed and diluted immediately prior to measurement.

Plasmonic interferometers: Fabrication and optical characterization

Arrays of groove-slit-groove (GSG) plasmonic interferometers with arm lengths p₁=700 μm and p₂=7–10 μm in steps of 25 nm (as well as single slit devices for normalization purposes) were fabricated on a 300-nm-thick Ag film deposited on top of a 1 inch by 1 inch quartz slide using focused ion beam (FIB) milling. Each slit (groove) was 100 nm (200 nm) wide and 10 μm long. A 5-nm-thick, optically transparent layer of Al₂O₃ was deposited on top of the milled films using atomic layer deposition (ALD) to prevent the silver from undergoing chemical modification in the presence of the buffer solution. A collimated beam of white light (λ=400–800 nm) illuminates the plasmonic interferometer at normal incidence. A 40x objective lens (numerical aperture NA=0.6) was used to collect the transmitted light,
which is then dispersed by a single-grating spectrograph and detected by a charge-coupled device (CCD) camera. The spectral resolution of the optical setup was ~0.4 nm; the number of counts at each wavelength and number of acquired spectra per experiment were adjusted to ensure a statistical error <0.1% in the measured transmitted intensity. An initial reaction mixture of the AR/GOx/Glucose assay was reacted with various concentrations of glucose (0–250 μM) in the dark for 50 min to allow the reaction to go to full completion. The reacted solution was then delivered to the plasmonic interferometer via a polydimethylsiloxane (PDMS) microfluidic channel (2-cm long×1-cm wide×70-μm high) using a syringe pump at a flow rate of 150 μL/min.

**UV-Visible absorption spectroscopy**

Spectroscopic measurements were performed with a dual-beam UV-Visible Cary 500 Spectrophotometer (Agilent Technologies, Chesterfield, MO) using a quartz cuvette with a path length of L=0.2 cm. The same initial reaction mixture that was used with the plasmonic interferometers was used for both the optical characterization of resorufin and kinetic experiments. For the optical characterization of resorufin, the reaction mixture was initiated with 100 μL of glucose at a concentration of 10.0±0.4 μM, 25±1 μM, 50±2 μM or 100±4 μM, reacted for 50 min in the dark; the transmittance was measured for incident wavelengths between 450 and 700 nm. For the kinetic experiments, the reaction was initiated with the addition of various concentrations of H₂O₂ or glucose (both in 100 μM solutions). The reaction was monitored at the wavelength where resorufin exhibits maximum absorption (λ_\text{\text{max}}=571 nm) in 0.1- or 1-s intervals. All experiments were performed in triplicate at standard temperature (25°C) and pressure (760 mm Hg); the concentration of dissolved oxygen ([O₂]) was assumed to be constant and equal to 261 μM (calculated using Henry’s law) [46]. All samples were illuminated with a 50 W tungsten light source; the light intensity was measured every 1 s for 2000 s for each reaction and each condition was studied in triplicate.

**Selectivity test for glucose in artificial saliva**

Modified Fusayama artificial saliva (MFAS) was prepared to determine the selectivity for glucose in the AR/GOx/Glucose assay. This mixture contains NaCl (6.8 mM), KCl (5.4 mM), CaCl₂·2H₂O (5.4 mM), NaH₂PO₄·H₂O (5.0 mM), KSCN (3.1 mM), Na₂S·9H₂O (6.9 μM) and urea (16.7 mM) at a pH of 7.4 [47]. A 600-μL solution for measuring glucose concentration was prepared by mixing an initial reaction mixture of the AR/GOx/Glucose assay with 100 μL of MFAS containing 100 μM glucose. Diluting the MFAS solution by 7× increases the solubility of urea and the salts found in the mixture, while still allowing the assay reactions to occur. Control experiments used the same reaction mixture initiated with either 100 μL of the modified Fusayama artificial saliva without glucose or 100 μL of 50 mM sodium phosphate buffer with 100 μM glucose. The transmitted light intensity was measured every 1 s for 2000 s for each reaction and each condition was studied in triplicate.

**Appendix B: Guide to nomenclature**

The plasmonic cuvette merges two disparate fields into one device: dye chemistry and plasmonic interferometry. Because of the various conventions and standards that exist in the realms of chemistry and physics, it is important to be clear with the notation in this manuscript.

There are two equivalent conventions for discussing the amount of molecules in a solution. Concentration c, in units of moles/volume (called a molar, M), is typically used by chemists. Density ρ is typically used by physicists, and is expressed in (length)^3. The conversion factor from one to the other is:

$$\rho = cN_A$$  \hspace{1cm} (6)

where $N_A$ is Avogadro’s constant ($N_A=6.022\times10^{23}$ mol⁻¹).

For both cuvettes mentioned in this report (plasmonic and UV-Vis), the quantity that the system measures directly is the spectral transmittance, defined as:

$$T = I_T / I_{\text{ref}}$$  \hspace{1cm} (7)

where $I_T$ is intensity transmitted through the device and $I_{\text{ref}}$ is the reference intensity (i.e., the intensity of the reference beam for the standard UV-Vis cuvette, and the light intensity transmitted through an isolated slit for a plasmonic interferometer). $I_T$ and $I_{\text{ref}}$ refer to the background-subtracted signals. For a UV-Vis cuvette, under the assumption that no significant reflection occurs, the law of conservation of energy states that:

$$A = 1 - T$$  \hspace{1cm} (8)

where A is the absorbance. The Beer-Lambert law defines a closely related quantity, the chemical absorbance (sometimes called absorptance) $A_b$:

$$A_b = -\log_{10}(T)$$  \hspace{1cm} (9)
A is typically used by physicists, whereas \( A_b \) is typically used by chemists. Although they both share the property of \( 0 < A, A_b < 1 \), they are mathematically distinct.

Next, the absorption coefficient \( \alpha \) is defined. This quantity, in units of (length)\(^{-1}\), depends on the intrinsic properties of the absorbing molecule and its concentration in the solution. The first principles definition is:

\[
T = e^{-\alpha L} \rightarrow \alpha = -\frac{\log(T)}{L}
\]  

(10)

where \( L \) is the path length of the cuvette (in cm). This expression appears in both physics and chemistry. However, it is often useful to define a quantity that can help quantify absorption and absorbance, but does not depend on the concentration of a species with a solution (that is, it is purely an intrinsic property of the absorbing molecule). Once again, physics and chemistry diverge on this point. Chemists prefer to use the extinction coefficient \( \varepsilon \) (also known as the molar absorptivity), which is defined as:

\[
\varepsilon = A_b / cL
\]  

(11)

and is expressed in (molar)\(^{-1}\) (length)\(^{4}\). Physicists prefer to use the absorption cross-section \( \sigma \) (in units of (length)\(^2\)), which is defined as:

\[
\sigma = \alpha / \rho
\]  

(12)

There is a linear relationship between \( \varepsilon \) and \( \sigma \); either of these can be used to relate the time- and wavelength transmission to the measured concentration or density of the optically-active resorufin species. This relationship can be obtained by plugging Eq. (11) into Eq. (9):

\[
\varepsilon cL = -\log_{10}(T) \rightarrow c = \frac{\log_{10}(T)}{\varepsilon L}
\]  

(13)

This is Eq. (5) in the main text; the notation \([x]\) refers to the time-dependent concentration of the species within the bracket. Next, plug in the definition of \( T \) from Eq. (10):

\[
c = -\frac{\log_{10}(e^{-\alpha L})}{\varepsilon L} = \frac{c \log_{10}(e)}{\varepsilon}
\]  

(14)

Using the definition of \( \alpha \) from Eq. (12):

\[
c = \frac{\rho \log_{10}(e)}{\varepsilon}
\]  

(15)

Recalling that \( \log(y) = \frac{1}{\log(x)} \) and re-arranging Eq. (15) gives:

\[
\sigma = \frac{c \log_{10}(1)}{\rho} \varepsilon
\]  

(16)

This is Eq. (3) in the main text. Finally, we plug in Eq. (6) to obtain the relationship between \( \varepsilon \) (extinction coefficient) and \( \sigma \) (absorption cross section):

\[
\sigma = \frac{\log_{10}(e)}{N_A} \varepsilon = (3.824 \times 10^{-21} \text{ mol}) \varepsilon
\]  

(17)

### Appendix C: Spectroscopic characterization of the absorption properties of resorufin

**Figure 6**  
(A) Experimental spectra of the absorption coefficient, \( \alpha \), for four different concentrations of resorufin in a 50 mM sodium phosphate buffer solution (pH 7.4) using a 0.2-cm path length cuvette. (B) Absorption coefficient as a function of the density of absorbing resorufin molecules (\( \rho \)) or the molar concentration of resorufin (\( c \)) at \( \lambda_1 = 530 \text{ nm} \) (blue circles), \( \lambda_2 = 571 \text{ nm} \) (red squares) and \( \lambda_3 = 590 \text{ nm} \) (purple triangles). This plot was obtained by taking a cross-section of the curves in panel (A) at \( \lambda_1, \lambda_2 \) and \( \lambda_3 \). (C) The slope of a linear fit of curves in panel B represents the absorption cross-section of a resorufin molecule (\( \rho \)) at a specified wavelength, as indicated by Eq. (12). This process was repeated for the entire range of wavelengths (450 nm < \( \lambda \) < 700 nm) in steps of 1 nm to obtain the functional dependence of \( \sigma \) versus \( \lambda \) reported in panel C.
Appendix D: Determination of rate constant for Reaction 3, $K_3$

Figure 7  Concentration of resorufin plotted as a function of time (symbols) in steps of 0.1 s, obtained from transmission measurements (at $\lambda_{\text{max}}=571$ nm). The solution contains $9.8 \pm 0.2 \mu$M resorufin and $8.8 \pm 0.2 \mu$M $H_2O_2$, with either 0 nM, 5.5 ±0.1 nM, 27.5±0.6 nM, or 55±11 nM HRP. The transmission data was plugged into Eq. (5) to find the concentration of resorufin. Experimental data points are reported every 5 s for clarity; the confidence band indicates the error for all data points. The measurements were repeated three times at each [HRP] value; the data in the figure shows the average value. The fits (solid lines) are obtained using a pseudo-first-order kinetics model (Eqs. 4E–4F) with $K_1=0$ and $K_2=0$. The best agreement between experimental data and the mathematical model corresponds to the case where [HRP] was 5.5±0.1 nm. Therefore, this concentration of HRP was used for all other kinetic experiments in the paper – $K_3$ was found to be $56 \pm 7$ M$^{-1}$ s$^{-1}$ when averaged over the three trials at that particular concentration.

Appendix E: Determination of rate constant for Reaction 2, $K_2$

Figure 8  (A) Concentration of resorufin plotted as a function of time (symbols) in steps of 0.1 s obtained from transmission measurements (at $\lambda_{\text{max}}=571$ nm). The solution contains 280±6 μM AR, 5.5±0.1 nm HRP, and either 3.8±0.1 μM, 7.8±0.1 μM, or 15.5±0.3 μM $H_2O_2$. The transmission spectrum, along with Eq. (5), was used to find the concentration of resorufin. The measurements were repeated three times at each $[H_2O_2]$ value; the data in the figure shows the average value. Experimental data points are reported every 5 s for clarity; the confidence band indicates the error for all data points. The fits (solid lines) are obtained using a pseudo-first-order kinetics model (Eqs. 4C–4F) with $K_1=0$ and $K_3=56 \pm 7$ M$^{-1}$ s$^{-1}$ as previously determined from Figure 7, Appendix D. (B) The average rate constant $K_2$ was determined for three independent trials at each concentration of $H_2O_2$ (the error bars are within the data points). The mean value of all trials and concentrations is $K_2=135 \pm 5$ M$^{-1}$ s$^{-1}$.

Appendix F: Determination of rate constant for Reaction 1, $K_1$

Figure 9  Determination of $K_1$. The mean value of three independent trials at each concentration of glucose is reported as $K_1=7.3 \pm 0.3$ M$^{-1}$ s$^{-1}$.
Appendix G: Simulation of concentrations of assay reagents as a function of reaction time

Figure 10  Concentration profile of all six reactants in the AR/GOx/Glucose assay simulated with: (A) $[\text{G}]_{t=0}=30.2\pm0.1\mu\text{M}$ and (B) $[\text{G}]_{t=0}=210\pm5\mu\text{M}$ as functions of time. G, Glucose; O$_2$, Oxygen; H$_2$O$_2$, Hydrogen Peroxide; AR, Amplex red; R, Resorufin; OIP, Optically Inactive Product. (C) Concentration profile of H$_2$O$_2$ production over time simulated at different initial glucose concentration ($[\text{G}]_{t=0}$) conditions. Even when the initial concentration of glucose is high, the H$_2$O$_2$ concentration never exceeds 10 $\mu$M at any instant of time.

Appendix H: Spectroscopic fingerprints of Resorufin

Figure 11  Spectral absorption of resorufin at different times after the reaction is initiated; each curve is normalized to its own maximum value. The insets show the raw absorption data, background corrected. In both panels, the mixture contains 280$\pm$6 $\mu$M AR, 5.5$\pm$0.1 nm HRP. Panel (A) includes 82.5$\pm$0.7 nm GOx and 50.0$\pm$0.9 $\mu$M glucose (Reactions 1, 2 and 3 occur); (B) includes 27.1$\pm$0.5 $\mu$M H$_2$O$_2$ (only Reactions 2 and 3 occur). The curves within each panel are practically identical at all times, clearly demonstrating that no other optically active product besides resorufin is being generated in the reactions.
Appendix I: Effects of illumination intensity on Amplex red

Figure 12  Effects of illumination intensity on the photo-oxidation of Amplex red. (A) Three neutral density filters – Filter A (transmittance=22%), Filter B (7.6%) and Filter C (0.85%) – were used to attenuate the illumination intensity of the visible light source of the UV–Vis spectrophotometer. (B) The resorufin concentration was determined for a reaction mixture containing 280±6 μM AR, 5.5±0.1 nM HRP and 27±0.5 μM H₂O₂ after 300 s in the dark (in gray) and after illumination every 0.1 s for 300 s. The illumination intensity was decreased with the use of neutral density filters. The experiments were repeated in triplicate. The data show that the illumination intensity does not significantly affect the final concentration of resorufin, which means that photo-oxidation of Amplex red is not occurring in the time scales and optical powers used in these experiments.

Appendix J: Dependence of rate constants on enzyme concentrations

Figure 13  (A) Relationship between $K_1$ and concentration of GOx in Reaction 1. The data points refer to experimental measurements of $K_1$ performed in triplicate (average value reported). The data was fit against the following relationship:

$$K_1 = \frac{C_1[GOx]}{C_2+[GOx]}$$  (18)

where $C_1$ and $C_2$ are fitting parameters. It was found that $C_1=48.5±4.2 \text{ M}^{-1} \text{s}^{-1}$ and $C_2=(4.5±0.9)×10^{-7} \text{ M}$. Although this curve resembles the functional form of the Michaelis-Menten curve, it needs to be emphasized that Michaelis-Menten model is used to find the relationship between the rates of product generation (i.e., velocity $v$) as a function of substrate concentration ($[S]$). Instead, Eq. (18) is being used to determine the reaction rate ($K_1$) as a function of enzyme concentration ([GOx]) [48]. The Michaelis-Menten model is not directly applicable to this data, because one of the foundational assumptions used to simplify the model – that the initial substrate concentration is much greater than the concentration of
enzyme – is not applicable here. In this system, the initial concentration of substrate (i.e., glucose) is at most only two orders of magnitude higher than the enzyme concentration [GOx] with [G] rapidly declining as it is converted into product (B). Extracted $K_v$ values as a function of HRP concentration indicates a linear dependence with a slope of $K_v = (1.45 \pm 0.04) \times 10^{10} \text{ M}^{-1} \text{s}^{-1}$. Results are reported in triplicate.

References


