

On the Possibility of Glucose Sensing Using Boronic Acid and a Luminescent Ruthenium Metal-Ligand Complex

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We describe a new approach to optical sensing of glucose based on the competitive interactions between a ruthenium metal ligand complex, a boronic acid derivative and glucose. The metal-ligand complex [Ru(2,2'-bipyridine)₂(5,6-dihydroxy-1,10-phenanthroline)](PF₆)₂ at pH 8 forms a reversible complex with 2-toluyboronic acid or 2-methoxyphenyl boronic acid. Complexation is accompanied by a several-fold increase in the luminescent intensity of the ruthenium complex. Addition of glucose results in decreased luminescent intensity, which appears to be the result of decreased binding between the metal-ligand complex and the boronic acid. Ruthenium metal-ligand complexes are convenient for optical sensing because their long luminescent decay times allow lifetime-based sensing with simple instrumentation.

KEY WORDS: Glucose sensing; boronic acid; metal-ligand complex.

INTRODUCTION

Diabetes is a chronic disease affecting 100 million people worldwide. Convenient methods to measure glucose are needed for control of blood sugar in diabetics. It is now clear that tight control of blood glucose is needed to prevent the long-term health consequences of diabetics [1]. These complications include nerve damage, heart disease, and blindness. At present the only method to measure blood glucose is using a finger stick. The pain and inconvenience of this method results in infrequent measurements and poor control of blood glucose.

Considerable research effort has been directed toward finding a method for non-invasive measurements of blood glucose [2]. These methods include mid-infrared

and near-infrared spectroscopy, optical rotation, and fluorescence methods. The fluorescence methods can be divided into two classes, those based on proteins that bind to sugars [3–11] and those based on the interaction of boronic acid with sugars and glycols [12–18]. These glucose-sensitive probes display changes in fluorescence intensity in the presence of glucose, which is typically the result of a change in the extent of photoinduced electron transfer (PET). Sensing methods based on fluorescence probes are desirable because of their stability compared to protein-based sensors.

While methods are available to measure glucose in isolated samples, the goal of non-invasive measurement of blood glucose remains elusive. Hence, there has been increasing interest in minimally invasive methods. One promising approach is based on the use of interstitial fluid extracted from beneath the skin. It appears that this

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ABBREVIATIONS: bpy, 2,2'-bipyridine; BDA, 2-bromophenylboronic acid; dhph, 5,6-dihydroxy-1,10-phenanthroline; LED, light emitting diode; MBA, 2-methoxyphenyl boronic acid; MLC, metal-ligand complex; MLCT, metal-to-ligand charge transfer; PET, photoinduced electron transfer; TBA, 2-toluyboronic acid.

fluid tracks blood glucose [19]. Interstitial fluid can be extracted by several methods, sometimes combined with laser ablation of the stratus corneum [20–21]. Such methods are painless and can be performed at frequent intervals. Hence, there remains interest in developing convenient, non-consumptive and reversible glucose sensors to be used with these emerging methods.

In the present paper we extend the earlier studies of boronic acid–based glucose sensors [17–18] to include a metal-ligand complex (MLC). In recent years, MLCs have been developed as luminescent probes [22–27]. MLCs display long luminescent lifetimes, high photostability, and convenient visible absorption bands. Thus, a glucose-sensitive MLC may find use in portable devices for glucose measurements.

Our approach to measuring glucose is outlined in Scheme I. The MLC contains 5,6-dihydroxy-1,10-phenanthroline, which is ionized at pH 8 (Step 1). The ionized diol can interact with boronic acid derivatives, which can alter the spectral properties of the MLC $[\text{Ru}(\text{bpy})_2(\text{dhph})]^{2+}$ (Step 2). Glucose is also known to interact with boronic acid and can remove boronic acid from the MLC (Step 3). We show that suitable spectral change occur according to Steps 1–3. Although the changes in Step 3 are minimal at this time for a practical

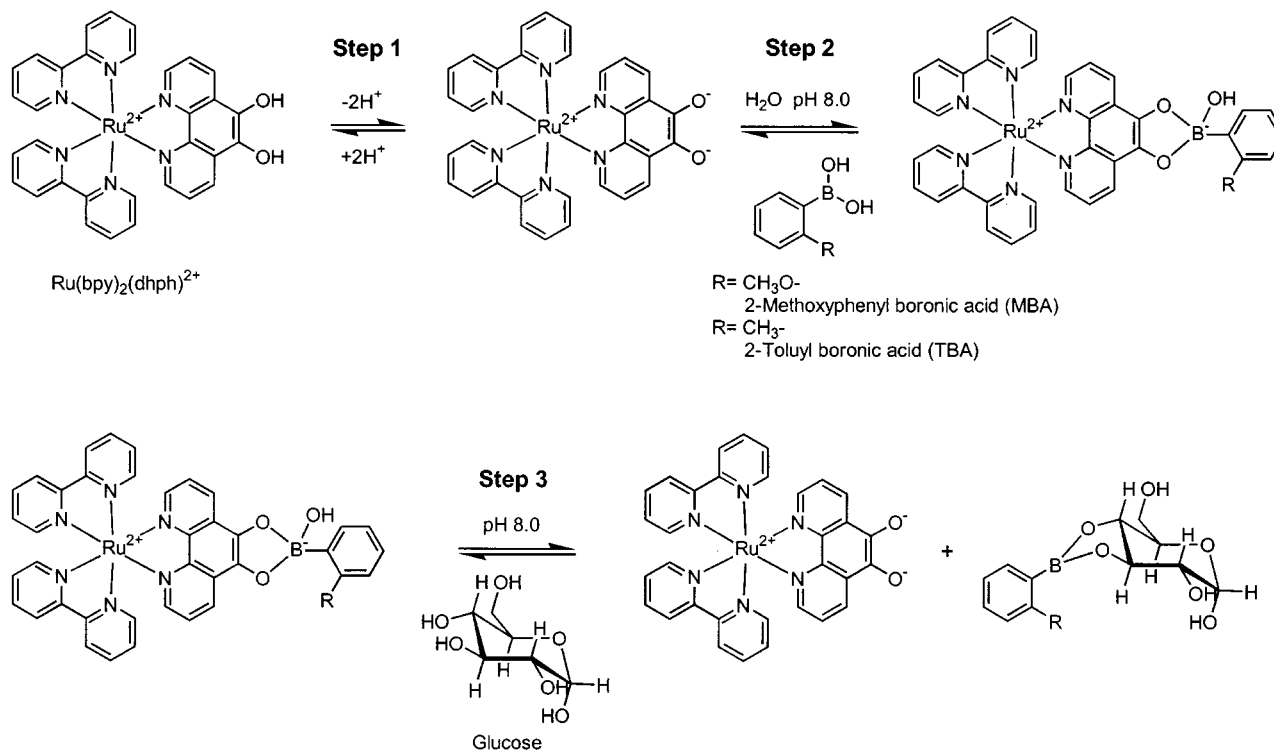
glucose sensor, this paper provides proof that this approach can be utilized for the development of a working sensor.

MATERIALS AND METHODS

Ruthenium trichloride 2,2'-bipyridine, 2-toluy boronic acid, and 2-methoxyphenyl boronic acid, solvents, and chemicals were purchased from Aldrich and used without further purification. Distilled water was used for all aqueous solutions. The starting material $\text{Ru}(\text{bpy})_2\text{Cl}_2$ and dhph ligand were prepared as described previously [28] with slight modifications.

Synthesis of $[\text{Ru}(\text{bpy})_2(5,6\text{-dihydroxy-1,10-phenanthroline})](\text{PF}_6)_2$

$[\text{Ru}(\text{bpy})_2(5,6\text{-dihydroxy-1,10-phenanthroline})](\text{PF}_6)_2$ was prepared by refluxing $\text{Ru}(\text{bpy})_2\text{Cl}_2$ with 5, 6-dihydroxy-1,10-phenanthroline in 1:1.2 ratio for 4 h in dimethylformamide (DMF). Upon completion of the reaction, the mixture was cooled to room temperature and then vacuum dried leaving a brown solid. This solid was dissolved in water and filtered. The aqueous supernatant



Scheme 1. Analytical scheme for sensing glucose based on competitive binding with $[\text{Ru}(\text{bpy})_2(\text{dhph})]^{2+}$ and boronic acid.

was saturated with ammonium hexafluorophosphate to precipitate the complex, which was separated by filtration. The product was washed successively with cold water and ether and dried in vacuum. The compound was dissolved again in a minimum amount of acetone and separated from solution by the addition of ammonium hexafluorophosphate. The solid was filtered, washed with cold water and ether, and dried. The resulting orange-brown powder was further purified by elution with acetone through an LH-20 column. The compound was characterized by elemental and mass spectroscopy.

Instrumentation

A commercial cross-correlation phase fluorometer (ISS, Urbana, IL) was modified to use a blue LED (Nichia NSPB500, Nichia Chemical Industries, Lancaster, PA) for the excitation source [29–31]. The LED was biased at 6.0 mA with a ILX Lightwave (Bozemann, MT) DX-3412 Precision Current Source through a picosecond pulse labs (Boulder, CO) 5580 bias tee. A Marconi Instruments (Allendale, NJ) 2022D signal generator supplied a modulation current + 6 dBm. Fluorescence emission was filtered to remove scattered excitation light by a single Andover (Salem, NH) 600FH90-50S 600-nm-long wave pass filter and detected by Hamamatsu (Bridgewater, NJ) R928 photomultiplier tube. Cross-correlation was achieved by modulating the photomultiplier tube bias with a second Marconi 2022D, also at + 6 dBm, amplified by a Mini-Circuits (Brooklyn, NY) ZHL-32A amplifier, for a total output of about + 30 dBm. A scattering solution of 1% Ludox (DuPont, Willmington, DE) colloidal silica was used as a reference. This scattered light was filtered by neutral density filters so that the amount of light detected was comparable to the emission of the sample.

Phase angles (φ) and modulations (m) were measured at eight frequencies spaced logarithmically ranging from 50 kHz–5MHz. These values were used by the least squares fitting program provided by ISS to find the best fit for one, two, or three discrete exponential decay lifetimes.

The frequency-domain intensity decay were fit to a single and multiexponential models. The analyses were performed with non-linear least square procedures [31]. The intensity decays were described by

$$I(t) = \sum_{i=1}^n \alpha_i e^{-t/\tau_i} \quad (1)$$

where α_i are the preexponential factors, τ_i are the decay times, and n is the number of exponential components. The mean decay time is given by

$$\bar{\tau} = \frac{\sum_i \alpha_i \tau_i^2}{\sum_i \alpha_i \tau_i} \quad (2)$$

Table I. Effect of pH on the Fluorescence Lifetime of $[\text{Ru}(\text{bpy})_2(\text{dhph})]^{2+}$

Sample	$\tau_1(\text{ns})$	f_1	$\tau_2(\text{ns})$	f_2	X_R^2	$\tau_{\text{ave}}(\text{ns})$
pH 4	325	0.98	51	0.02	2.8	319.5
pH 6	290	0.97	51	0.03	3.6	282.8
pH 8	285	0.96	41	0.04	2.7	275.2

RESULTS

The emission intensity of $[\text{Ru}(\text{bpy})_2(\text{dhph})]^{2+}$ decreases as the pH is increased from 4.3 to 8.0. This decrease is thought to be due to the ionization of the two hydroxyl groups (**Step 1**). The intensity decay is dominantly a single exponential with a mean decay time near 300 ns (Table I). The mean decay time decreases from 320 to 285 ns as the pH is raised from 4 to 8. The decay time near 300 ns is assigned to the metal-to-ligand charge transfer state (MLCT). At present we are unsure of the origin of the shorter decay time near 50 ns.

The emission intensity of $[\text{Ru}(\text{bpy})_2(\text{dhph})]^{2+}$ is strongly dependent on the presence of a boronic acid derivative. Addition of 2-toluyboronic acid (TBA) to the complex results in a 3-fold increase in intensity (Fig. 1). This increase in intensity could be partially reversed by addition of glucose (Fig. 2). The decrease in luminescence intensity occurs in the physiological range of blood glucose concentrations near 5mM. These results are marginal (~8%) but suggest that an MLCT probe such as

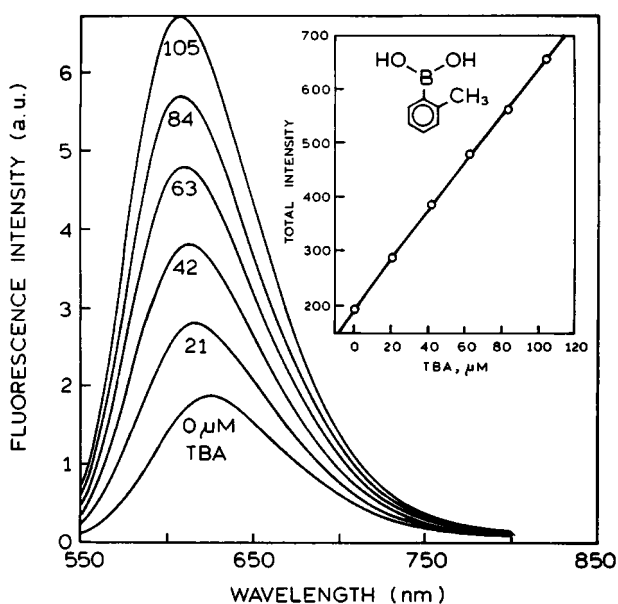


Fig. 1. Emission spectra of 45 μM $[\text{Ru}(\text{bpy})_2(\text{dhph})]^{2+}$ in the presence of increasing concentrations of TBA at pH 8.0.

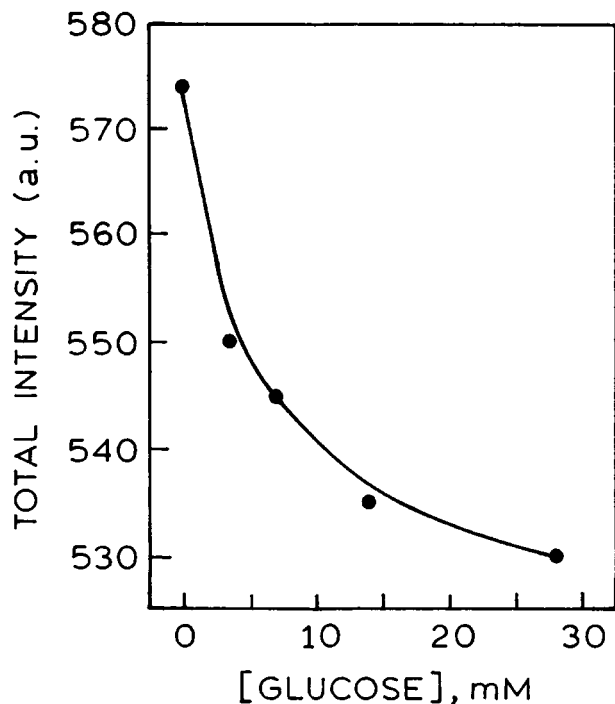


Fig. 2. Effect of increasing concentrations of glucose on the total intensity of $45 \mu\text{M}$ $[\text{Ru}(\text{bpy})_2(\text{dhph})]^{2+}$ in the presence of $45 \mu\text{M}$ TBA at pH 8.0.

$[\text{Ru}(\text{bpy})_2(\text{dhph})]^{2+}$ and a boronic acid can be used for optical sensing of glucose.

Addition of a different boronic acid, 2-methoxyphenyl boronic acid (MBA), results in a 5-fold increase in luminescence intensity of $[\text{Ru}(\text{bpy})_2(\text{dhph})]^{2+}$ (Fig. 3). Although this increase appears to be larger than that found for TBA (see Fig. 1), the normalized intensities show that the TBA has a larger effect than MBA (Fig. 4). TBA does not dissolve in water as well as MBA, which prevents us from doing additional measurements at comparable concentrations. This suggests weaker binding of MBA to the MLC. We initially thought that weaker binding may be desirable in that the MLC-boronic acid interaction may be more readily reversed by glucose. However, it became clear that the strength of glucose binding to the boronic acid should also be considered.

For an MBA concentration of $180 \mu\text{M}$, addition of glucose results in an 11% decrease in intensity of the MLC at 40 mM glucose. The plot (inset) suggests that saturation has not been reached and an even larger decrease can be expected at glucose concentrations higher than 40 mM . However, at the physiological glucose level, the change is only 4%. Although not favorable for our purposes, these results suggest that small changes in the boronic acid structure allows for selecting the range of measurable glucose concentration. In the future, we also

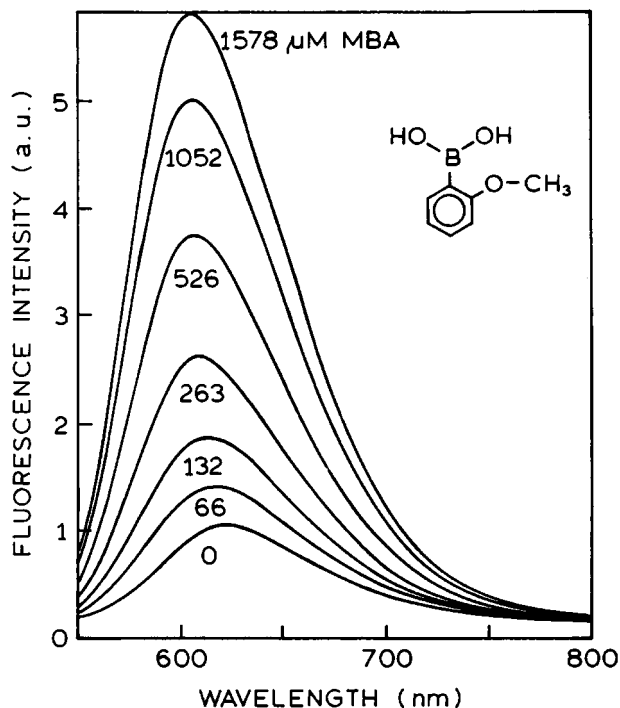


Fig. 3. Emission spectra of $45 \mu\text{M}$ $[\text{Ru}(\text{bpy})_2(\text{dhph})]^{2+}$ in the presence of increasing concentrations of MBA at pH 8.0.

foresee larger changes in intensity by optimizing the boronic acid structure, as well as the MLC-boronic acid ratio. The larger changes should allow more accurate measurement of the glucose concentration.

In previous reports we showed that phase-modulation lifetime measurements can be insensitive to the total intensity from the sample, which is a valuable property

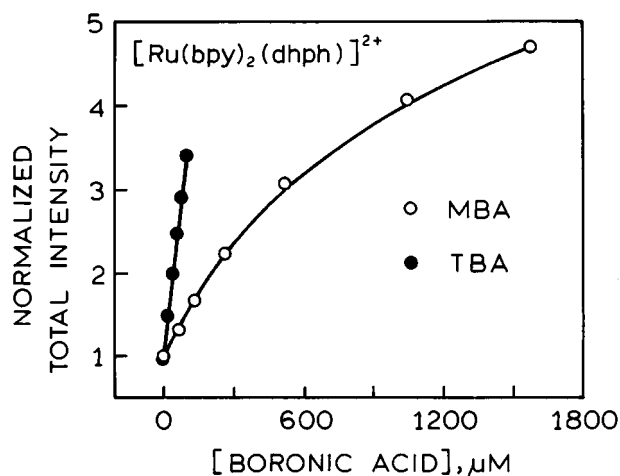


Fig. 4. Normalized total intensities of $45 \mu\text{M}$ $[\text{Ru}(\text{bpy})_2(\text{dhph})]^{2+}$ calculated from the spectra in Figs. 1 and 3, showing the relative reversibilities of the reaction of the TBA and MBA to $[\text{Ru}(\text{bpy})_2(\text{dhph})]^{2+}$.

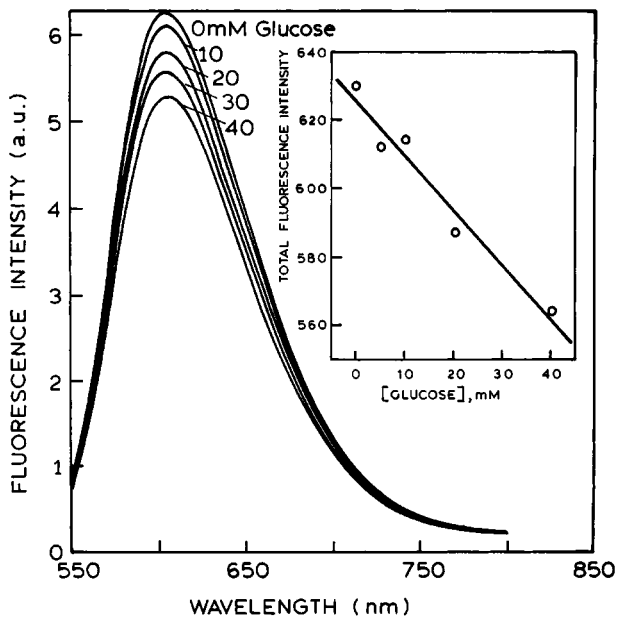


Fig. 5. Emission spectra of $45 \mu\text{M}$ $[\text{Ru}(\text{bpy})_2(\text{dhph})]^{2+}$ + $180 \mu\text{M}$ MBA with increasing glucose concentrations at pH 8.0. Insert: Intensities as a function of glucose concentration.

for medical uses of luminescence sensing [32]. Hence, we questioned whether our MLC could be used as a lifetime-based sensor for glucose. Frequency-domain intensity decay of $[\text{Ru}(\text{bpy})_2(\text{dhph})]^{2+}$ are shown in Fig. 6. Addition of MBA results in a substantial shift of the frequency responses to lower frequencies. This shift is the result of an increase in the mean decay time of $[\text{Ru}(\text{bpy})_2(\text{dhph})]^{2+}$ from 285–408 ns (Table II).

The changes in lifetime upon complexation between the MLC probe and MBA suggests the use of lifetime-

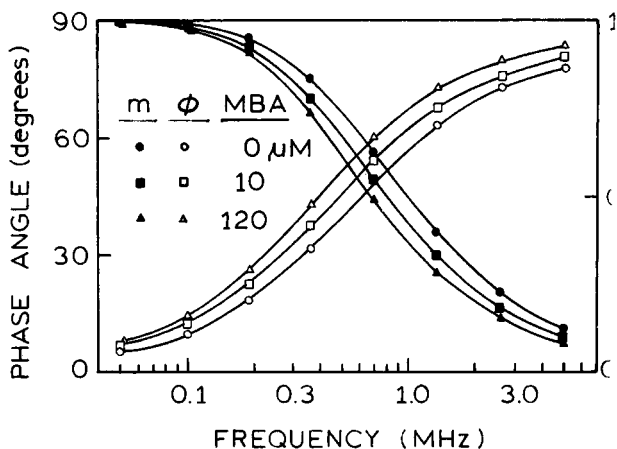


Fig. 6. Frequency-domain intensity decays $45 \mu\text{M}$ $[\text{Ru}(\text{bpy})_2(\text{dhph})]^{2+}$ in the presence of increasing concentrations of MBA at pH 8.0.

Table II. Fluorescence Lifetimes of $[\text{Ru}(\text{bpy})_2(\text{dhph})]^{2+}$ with Increasing Concentrations of Methoxyphenyl Boronic Acid at pH 8.0

MBA, (mM)	τ_1 (ns)	f_1	τ_2 (ns)	f_2	χ^2_R	τ_{ave} (ns)
0	285	0.96	41	0.04	2.7	275.2
0.132	352	0.97	50	0.03	2.8	343.0
0.263	372	0.98	46	0.02	2.7	365.5
0.526	391	0.99	37	0.01	1.9	387.5
1.052	402	0.99	37	0.01	2.2	398.4
1.578	408	0.99	31	0.01	2.4	404.2

based sensing. Unfortunately, we were unable to observe useful changes in the phase and modulation upon addition of glucose to the MLC-MBA complex. This is another aspect that needs to be addressed in order to make this method practical for glucose sensing. MLC probes with greater sensitivity to boronic acids will be designed and synthesized.

FUTURE PLANS

We have shown here that a glucose sensor based on a metal-ligand complex and boronic acid is feasible. One advantage of MLC is that the probe can be excited with a blue light-emitting diode (LED). The output of these LEDs is well matched to the MLCT transition near 450 nm. The optical output of LED can be easily modulated near 1 MHz [29–31], so that this solid-state light source could be used with steady-state or lifetime-based sensing.

We have not been able to observe lifetime changes in this system as yet. Nevertheless, the glucose-dependent intensity change of $[\text{Ru}(\text{bpy})_2(\text{dhph})]^{2+}$ can also be useful with recently developed methods for optical sensing. In several recent reports we described sensors that contained internal reference fluorophores. A change in intensity results in a change in modulation, which in turn can be used to measure the analyte concentration [33–35]. We also showed that one could use the polarization of the combined emission from a stretched-oriented reference film and the sensing fluorophore [36–37]. This method can be applied with any sensing fluorophore that displays a change in intensity. Polarization sensing can also be accomplished with visual detection [38]. One can readily imagine point-of-care devices based on such simple schemes for sensing. Thus, the glucose-dependent intensity change of our metal-ligand complex can be used with several methods for optical sensing of glucose.

Lastly, our results serve as proof of principle that glucose sensing can be achieved with a boronic acid and a luminescent MLC. There is a need to optimize the

boronic acid structure in order to make the competitive assay of glucose more sensitive. A quencher or acceptor group may also be attached to the boronic acid for a FRET-based sensor. This is also true for the MLC. An MLC that is more sensitive to the binding of boronic acid needs to be synthesized. We also foresee the MLC covalently linked to the boronic acid.

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