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Buffer and sugar concentration dependent fluorescence response of a BODIPY-based aryl monoboronic acid sensor

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ABSTRACT

The emission intensity response from a BODIPY-based aryl monoboronic acid was found to depend on the employed buffer at physiological pH (7.4). The structural prerequisites of the bound monosaccharides showed a clear discrimination between D-glucose and D-fructose. The different response behavior was ascribed to a major difference in the structural features of the arylboronate–monosaccharide complexes, under the employed buffered conditions. Supporting excitation measurements were found to correlate nicely with the emission experiments.

Continuous blood glucose monitoring offers great benefits in the treatment of diabetes in comparison to intermittent monitoring. Constant surveillance gives the diabetic patient the ability to monitor fluctuations in blood glucose, which limits the long term consequences of hyperglycemia. Another advantage is warning against hypoglycemia.[1–3] The same safety and control cannot be offered by conventional intermittent monitors. These are also inconvenient, due to the requirement of a blood sample several times a day.[4]

Intermittent monitoring is invasive, whereas continuous monitoring is semi-invasive or non-invasive. Semi-invasive glucose monitors generally provide less discomfort in comparison to their invasive counterparts. This is because the monitoring is performed through the skin.[5,6]

The elusive goal is to develop a reliable non-invasive approach utilizing, for example, monitoring tears, saliva, or sweat.[7–9] This kind of monitoring does not require a blood sample or a surgical procedure.

The small size of arylboronic acids[10–15] compared to other classes of carbohydrate binders, such as lectins[16] and artificial macrocycles,[17,18] enable them to be incorporated into larger structures, such as proteins, without altering the physical properties dramatically.

Furthermore boronic acids generally form cyclic esters, with *cis*-1,2-diols or 1,3-diols in aqueous media. These are formed rapidly and reversibly and are likely to change the photophysical properties of an arylboronic acid dye.

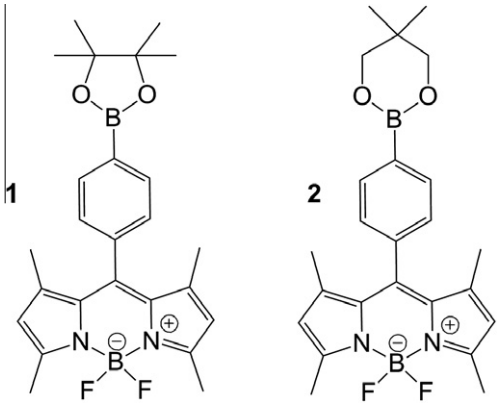

Figure 1. Our pinacol ester, **1**, and the neopentyl ester, **2**, prepared by DiCesare and Lakowicz.[20]

Arylboronic acids with displacement constants around 10–20 mM for D-glucose are desired, because blood glucose fluctuates between 2 and 30 mM in diabetes patients.[19] The maximum sensitivity is achieved when K_d is in the middle of the binding curve.

Sensor **1** is a pinacol ester analogue of a neopentyl ester, **2**, previously synthesized and evaluated by DiCesare and Lakowicz (Fig. 1).[20] Lakowicz's and Shinkai's groups discovered that the

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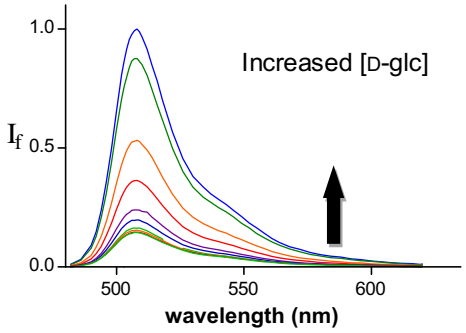

Figure 2. The significant increase in the emission intensity of **1**, upon addition of D-glucose from 0 to 275 mM in a 52.3 w/w% methanolic phosphate buffer.

binding of the boronic acid ester was independent of the nature of the ester, due to rapid ester exchange in boronic acid esters.[21,22]

Our research showed that compound **1** exhibited a different emission and excitation response pattern upon titration with D-glucose and D-fructose, employing a saline buffer (10 mM phosphate, 2.7 mM KCl and 137 mM NaCl), a phosphate buffer, and a methanolic phosphate buffer at physiological pH (7.4). These findings are crucial for understanding how the nature of the buffered solution affects the respective monosaccharide binding affinities.

Fluorometric evaluation of the complexes formed between **1** and D-glucose or D-fructose, showed a significant intensity increase in emission upon increasing the concentration of the two saccharides. The observed response for D-fructose was, as expected, significantly higher than the response for D-glucose. D-Fructose generally shows stronger binding to aryl monoboronates, due to the tridentate binding mode,[23] whereas D-glucose exhibits bidentate binding.[24]

However, selective recognition of D-glucose over other polyols is desired. D-Glucose is the major carbohydrate present in human blood, that is [D-glc] ≈5 mM,[25] compared to D-fructose; [D-frc] <0.5 mM, even after a fructose-rich meal.[26,27]

Additional excitation measurements revealed a significant increase in intensity upon augmenting the saccharide concentration, where the spectrum of excitation matches the spectrum of absorption. The response in fluorescence emission and excitation was further shown to depend on the employed buffer. A significantly higher response in excitation and emission intensities was observed in a methanolic phosphate buffer in comparison to the saline buffer and the free phosphate buffered solutions at pH 7.4. These results are interesting because the effect of the employed buffer is usually ignored. The saline buffered system is thought to be a better approach toward mimicking physiological media in comparison to the phosphate buffer.[28] However, the binding affinity toward D-fructose and D-glucose was found to be slightly different in the two buffers, with decreased glucose selectivity in the saline buffer. The results obtained in the phosphate buffers correlate well with the previous studies performed by DiCesare and Lakowicz.[20]

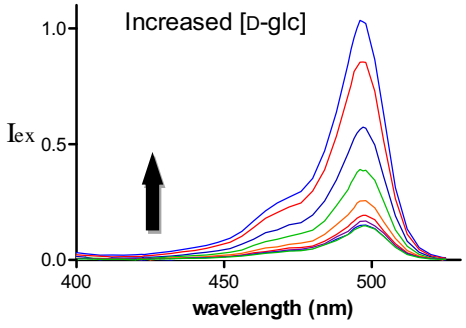

Figure 3. The significant increase in absorption of **1**, upon addition of D-glucose from 0 to 275 mM in a 52.3 w/w% methanolic phosphate buffer.

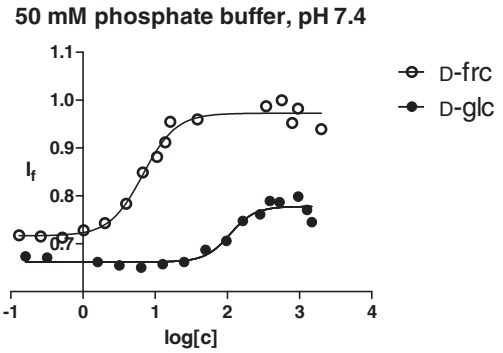

Figure 4. Curve fit using GraphPad Prism 5.0. The measured emission intensity was plotted against the logarithm of the concentration data (in mM), using sigmoidal dose response (variable slope). Compound **1** was used at 0.53 μM in the 50 mM phosphate buffered solution at pH 7.4.

Pinacol ester, **1**, was employed without deprotection, since the hydrolysis was expected to be complete in water.[21,22]

A significant increase in the fluorescence intensity of **1** was observed upon increasing the concentration of D-glucose or D-fructose, in all five buffer systems at pH 7.4. The increase in emission intensity was found to be more profound for D-fructose in all the employed buffer systems. The increase in the 52.3 w/w% methanolic phosphate buffer for both saccharides was superior to the other employed buffers. Emission curves for the increase in the 52.3 w/w% methanolic phosphate buffer are shown in Figure 2, and the corresponding increases in excitation are shown in Figure 3.

The sigmoidal curves for increase in emission intensity upon titration of compound **1**, with D-glucose and D-fructose in the 50 mM phosphate buffer system at pH 7.4, are depicted in Figure 4. Sigmoidal curves for binding in a 52.3 w/w% methanolic phosphate buffer are depicted in Figure 5.

Additional excitation and emission spectra were recorded in order to make corrections for light scattering of saccharides. The excitation and emission caused by light scattering of D-fructose and D-glucose at high saccharide concentrations were insignificant compared to the excitation and emission intensities in the buffered systems with **1**, that is less than 1% impact.

The increase in emission intensity, upon addition of sugars to a solution of **1** may be a consequence of formation of the arylboronate of **1**. This is favored by a decrease in the pK_a of **1** upon saccharide binding.[20] Formation of the boronate located at the *meso* aryl-substituent in **1**, can lead to a cessation of oxidative quenching

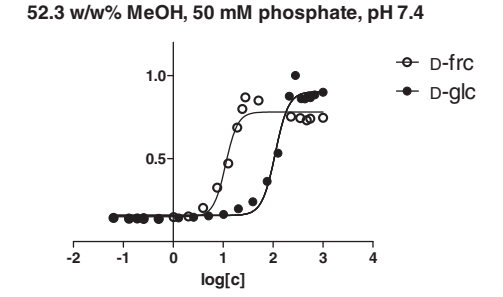

Figure 5. Curve fit using GraphPad Prism 5.0. The measured emission intensity was plotted against the logarithm of the concentration data (in mM), using sigmoidal dose response (variable slope). Compound **1** was used at 0.53 μM in 52.3 w/w% MeOH, 50 mM phosphate, pH 7.4.