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Glucose Sensing via Aggregation and the Use of "Knock-Out" Binding To Improve Selectivity

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Supporting Information

ABSTRACT: Aggregates of an amphiphilic monoboronic acid bearing a hydrophobic pyrene fluorophore were employed for highly modulating, sensitive, and selective ratiometric fluorescent sensing of glucose in aqueous solution. The selectivity for glucose was improved by "knock-out" binding of fructose by phenylboronic acid.

There is a clear and unmet clinical need for improved glucose monitoring.[1] Synthetic receptors (chemosensors) are at the fore in providing potential solutions. Toward that goal, boronic acids have been explored as synthetic receptors for saccharides such as glucose.[2] State-of-the-art boronic acid-based glucose-selective sensors rely on synthetically challenging receptors.[3] The approach has been used to great effect by us[4] and others.[5] However, no significant breakthroughs in sensitivity or selectivity have been made in over 17 years.

It is generally accepted that monoboronic acid derivatives display higher sensitivity and selectivity for fructose over glucose.[6] While improved glucose selectivity can be achieved through synthetically elaborate receptors bearing multiple boronic acid groups,[4b,7] a simpler option would be to develop novel sensing regimes to improve the inherent selectivity of simpler monoboronic acid systems. Toward that goal, we have been exploring the use of simple monoboronic acid receptor ensembles. Here we report the preparation and aggregation behavior of an amphiphilic monoboronic acid as a unique manifold for glucose-selective sensors.

The amphiphilic monoboronic acid (**1**, Figure 1) described in this report displays a selective ratiometric fluorescent response toward glucose. A monoboronic acid amphiphilic receptor containing a pyrene fluorophore was prepared for two reasons: (1) the formation of a long-wavelength emissive excimer at high bulk or local concentration[8] allows ratiometric sensing (the ratio of the two independent emission peaks is concentration independent, resulting in a calibration free system), and (2) the hydrophobic nature of pyrene promotes amphiphilic behavior of the system.

Compound **1** was designed to contain a cationic pyridinium moiety to provide a cation−π interaction capable of quenching the fluorescence of the pyrene monomer.[9] Compound **1** was readily prepared in two steps (Supporing Information). Figure 2

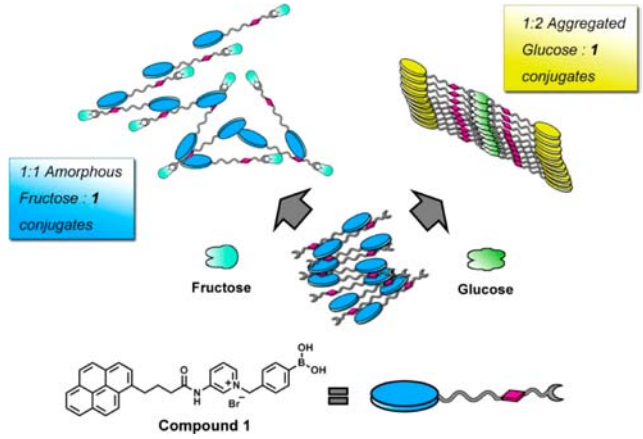

Figure 1. Cartoon illustrating the 1:1 fructose complex and the 1:2 glucose aggregate formed respectively with **1**.

shows the fluorescence spectra of **1** in alkaline carbonate buffer at pH 10.0 in the presence of glucose and fructose. Compound **1** exhibits a structured pyrene monomer fluorescence around 390 nm. The presence of glucose leads to the development of an excimer emission at 510 nm, which increases in intensity with added glucose, while the monomer emission is only slightly enhanced (Figure 2a). In sharp contrast, fructose produces only a modest enhancement of the monomer fluorescence and no excimer emission (Figure 2b). The lowest concentration of glucose that can induce excimer emission of **1** was 10 μM (Figure S1), while galactose and mannose, which contain two *cis*-diol moieties,[10] can only induce a small excimer emission (Figure 2b inset and Figures S2 and S3). Therefore, monoboronic acid compound **1** exhibits a highly selective, sensitive, and ratiometric fluorescent response toward glucose, in stark contrast to previous monoboronic acid receptors,[11] where the response is normally higher with fructose. In fact, the sensitivity observed for glucose with **1** is higher than that of most of the previously reported monoboronic acid-based chemosensors (Figures 2 and S2−S5). To achieve high sensitivity, signal amplification must be involved in the sensing of glucose using **1** under the measurement conditions. For **1**

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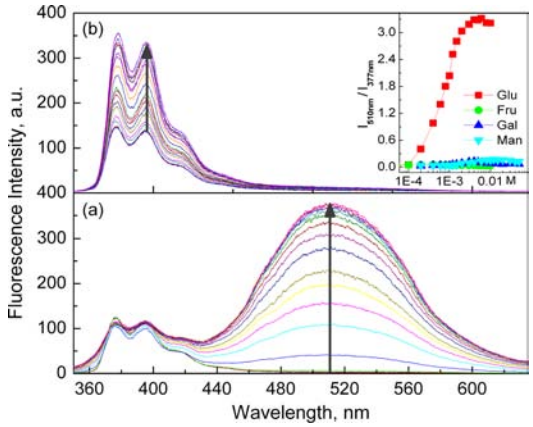

Figure 2. Fluorescence spectra of **1** in pH 10.0 carbonate buffer containing 2% (v/v) methanol in the presence of D-glucose (a) and D-fructose (b) over 0−10 mM. Inset in (b) shows the excimer-to-monomer intensity ratio as a function of saccharide concentration. [**1**] = 0.1 mM, λ_{ex} = 328 nm.

we attribute the signal amplification to saccharide-induced aggregation, as proposed in Figure 1. The structure of the aggregate glucose "monomers" is confirmed by the titration curve following the intensity at 510 nm, which can only be fitted using a 1:2 boronic acid to glucose stoichiometry (binding constant 2.0 × 10^6 M^{-2}, Figure S4).

The influence of aggregate formation on the fluorescence properties of **1** was probed as a function of pH. Profiles of the excimer-to-monomer emission intensity ratio and the diameter of **1** aggregates in solution in the absence and presence of glucose and fructose are shown in Figure 3. Figure 3a shows

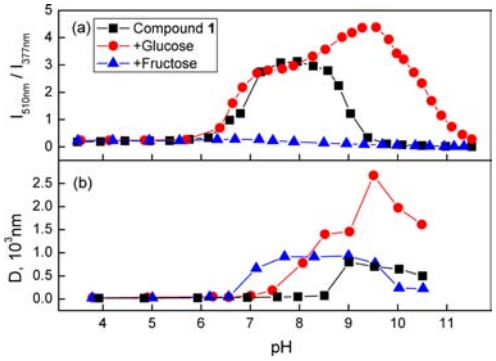

Figure 3. (a) Excimer-to-monomer emission intensity ratio (I_{510nm}/I_{337nm}) and (b) particle diameter (D) of **1** in the absence and presence of glucose and fructose. [**1**] = 0.1 mM, [glucose] = [fructose] = 1 mM. Particle diameter was measured by dynamic light scattering.

that at pH > 9.5, when the boronic acid group[12] exists as the boronate anion, no excimer emission was observed. Under these conditions, the addition of fructose did not lead to exicmer emission, but on the addition of glucose, a strong excimer emission was observed. This observation also correlates with the variation of particle/aggregate sizes observed in solutions of **1** (Figure 3b); i.e., in the presence of glucose, both a strong excimer emission and a relatively large particle size are observed. Interestingly, at pH 10.0, **1** forms smaller aggregates of diameters ca. 500 nm, but these do not result in excimer emission. Pyridinium cation−π interactions[9a−c] are likely to be a strong driving force for the aggregation of **1**. It is then reasonable to assume that 1:2 glucose:**1** boronates exist in larger aggregates than either the 1:1 fructose:**1** boronates or free **1** boronate, resulting in greater stacking of the pyrene fluorophores and producing a large excimer emission. (Figure 1). The 1:1 fructose:**1** conjugate displays enhanced pyrene monomer fluorescence due to the attenuated quenching efficiency of the pyridinium in the charge-neutral boronate. The absorption spectrum of **1** in the presence of fructose remains well structured, whereas in the presence of glucose it becomes less structured and broader (Figures S6 and S7), which also corroborates the different aggregation behaviors mediated by fructose and glucose.

To investigate the importance of the 2:1 complex in glucose sensing, we next looked at the response of **1** to mannitol, which is known for its high affinity for boronic acids and 2:1 complex formation.[11a] As expected, the addition of mannitol to **1** resulted in an enhanced excimer emission (Figure S8). However, when the concentration of mannitol was increased beyond 5 × 10^{-4} M^{-1}, the excimer emission decreased and monomer fluorescence was recovered, indicating that at higher mannitol concentrations the 1:1 complex with **1** predominates over the 2:1 complex. The importance of multivalent complexes of **1** and excimer fluorescence was further demonstrated by the strong excimer emission of **1** in the presence of the nucleoside guanosine (G), which is able to form a G-quadruplex in the presence of K^+.[13] Other nucleosides (adenosine, uridine, or cytidine) that do not form quadruplexes with potassium did not display excimer fluorescence (Figures 4 and S9).

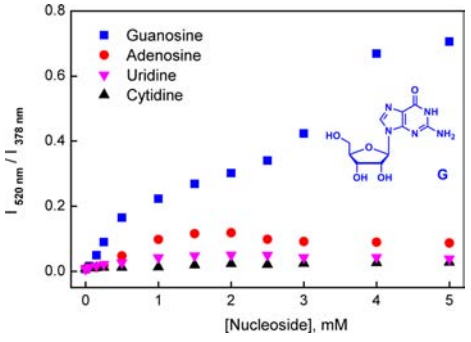

Figure 4. Plots of dual-fluorescence intensity ratio ($I_{520nm}/I_{378\ nm}$) of **1** against concentration of nucleosides guanosine, adenosine, uridine, and cytidine with KCl in pH 10.5 ethanolamine aqueous buffer. [**1**] = 0.1 mM, [KCl] = 20 mM, [ethanolamine] = 0.02 M.

Since formation of the **1** aggregate is very important in achieving selectivity and sensitivity for glucose, we set out to investigate what affect the degree aggregation has on selectivity. At a low compound **1** concentration of 0.05 mM, no aggregates are formed (Figure S10), and the sensitivity for glucose is much lower than that at [**1**] = 0.1 or 0.2 mM (Figure S1) when aggregates are formed. Aggregates of **1** are formed in solution at concentrations greater than 0.05 mM. Above that concentration, a selective response to glucose is observed, with higher concentrations of **1** producing better sensitivity. In a practical sense, [**1**] = 0.1 mM was chosen as a compromise between sensitivity and ease of aggregation control. The plot of excimer intensity of **1** is "S"-shaped with increasing glucose concentration (Figure S4 and legend), strongly suggesting a cooperative interaction[14] of glucose with **1**, resulting in enhanced levels of

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aggregation of the boronic acid. This may explain the observed signal amplification in glucose sensing and the apparent 1:2 interaction stoichiometry (Figure S4). In fact, the signal amplification may also contribute to the substantially improved selectivity for glucose over other saccharides (Figure 1b inset), which is again reflected in the relatively minor interference of fructose and other saccharides to glucose sensing. At a glucose concentration of 5 mM, only a 33% drop in the excimer-to-monomer intensity ratio of **1** was observed at 0.5 mM fructose, which is 50 times more concentrated than that found in human serum (Figure S11).[15] The interference observed with galactose and mannose was even lower. Using phenylboronic acid as a "knock-out" or mask for fructose, the interference of fructose could be improved even further such that only a 4% drop is observed at a fructose concentration of 5 mM when 10 mM phenylboronic acid is added (Figure 5). Phenylboronic acid has a

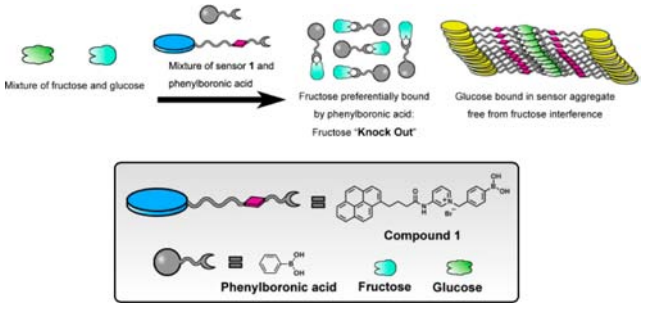

Figure 5. Cartoon illustrating the knock-out effect of an excess of phenylboronic acid. Phenylboronic acid binds to the fructose and, in so doing, allows **1** to exhibit a much better selectivity for glucose over fructose.

binding constant with fructose of K = 4365 and with glucose of K = 110, while **1** has a binding constant with fructose of K = 353 and with glucose of K = 1378. Clearly an excess PBA can effectively remove the fructose due to its higher fructose binding constant (12-fold) but will not interfere with the glucose binding of **1** due to its lower binding constant with glucose (12-fold).

Compound **1** is the most selective monoboronic acid sensor for glucose prepared to date. While Heagy has reported an excellent system (compound **2**), and while the sensor produces large spectroscopic changes with glucose and not with other saccharides such as fructose, the binding constant with glucose is significantly lower than that observed with **1** (Table 1). If we next compare our **1** aggregate with multiboronic acid receptors **3**–**5**, which have been specifically designed to be glucose selective, our system performs very well. Compound **1** has good selectivity for glucose (3.9-fold) over fructose. The largest selectivity for glucose over fructose is 43-fold for sensor **4**. For sensor **5** the binding constant with glucose is high but the selectivity is not reported because a binding constant could not be determined with fructose. It should be pointed out that being unable to determine a binding constant is not the same thing as sensor **5** not binding with fructose. For example, if we just considered the excimer emission (510 nm), we would not have been able to determine the binding constant for **1** with fructose; we needed to use the pyrene monomer emission to determine the fructose binding (377 nm). Cationic boronic acid–dye dissociation assays **6** and **7** have low to moderate selectivity (1.7-fold for **7**), but these systems are useful because they can produce large fluorescence enhancements (modulation) upon interaction with saccharides. A practically useful sensor requires not just selectivity and sensitivity, but also a large modulation of fluorescence when the concentration of glucose changes. When comparing **1** with the previously published systems (**2**–**7**), a modulation in fluorescence of 140-fold with glucose is significantly better than the 31-fold modulation observed for cationic boronic acid **6**.

In summary, we have developed a ratiometric fluorescent chemosensor based on an amphiphilic monoboronic acid that is highly selective and sensitive for glucose and also results in a very large modulation to changes in glucose concentration in aqueous solution. The presence of glucose leads to pyrene excimer emission, while its monomer emission remains more or less unchanged, whereas fructose results in a modest enhancement of the monomer emission. The positively charged sensor molecule containing a pyridinium moiety becomes zwitterionic at high pH and exists in aggregates. Glucose binding leads to more ordered aggregates of **1**, and since one glucose molecule could bind with two boronic acid groups, a more hydrophobic unit is formed, resulting in the pyrene fluorophores being brought into closer proximity and enabling the pyrene excimer emission. With fructose, however, the 1:1 binding stoichiometry results in a neutral zwitterionic boronate of higher hydrophilicity, destabilizing the aggregates of **1** and producing monomeric fructose boronates. We have shown that it is possible to use the aggregates of the monoboronic acid receptors to develop new, highly selective and sensitive receptors for the sensing of glucose in aqueous solutions. Compound **1** is a simple monoboronic acid that is easy to prepare yet achieves enhanced glucose selectivity via aggregation into a new sensor manifold resulting in a paradigm shift for glucose sensor design. With this work we have also demonstrated for the first time the concept of competitive "knock-out" of the fructose interference by adding phenylboronic acid to the sensing ensemble. With **1** we have stripped down molecular complexity while achieving optimized functionality to produce a state-of-the-art glucose-sensing

Table 1. Binding Constants (K, M^{-1}) of Various Sensors with Boronic Acid for Monosaccharides[a]

	monoboronic acid		diboronic acid			cationic boronic acid	
saccharide	**1**[b]	**2**[16]	**3**[17]	**4**[3b]	**5**[3a]	**6**[c,18]	**7**[19]
D-fructose	353 ± 21 (2.39)	480 (0.7)	320 (7.5)	34 (3)		5.0×10^4 (51)	1100 (2.6)
D-glucose	1.9×10^6,[c] 1378 (140)[d]	38 (0.5)	4000 (7.5)	1472 (8)	4.0×10^4 (0.5)	33 (31)	1900 (2.0)
D-galactose	24 ± 3 (1.73)		160 (6.0)	30 (3)	100 (0.7)	167 (68)	180 (2.1)
D-mannose	77 ± 9 (1.93)				62.5 (0.8)		
K_{Glu}/K_{Fru}	3.9	0.08	13	43		0.00066	1.7

[a]Number in the parentheses denotes maximum fluorescence change. [b]λ_{em} = 510 nm for glucose; λ_{em} = 377 nm for other saccharides. [c]K (M^{-2}) was obtained by assuming boronic acid:saccharide = 1:2. [d]The square root of K is shown for direct comparison.

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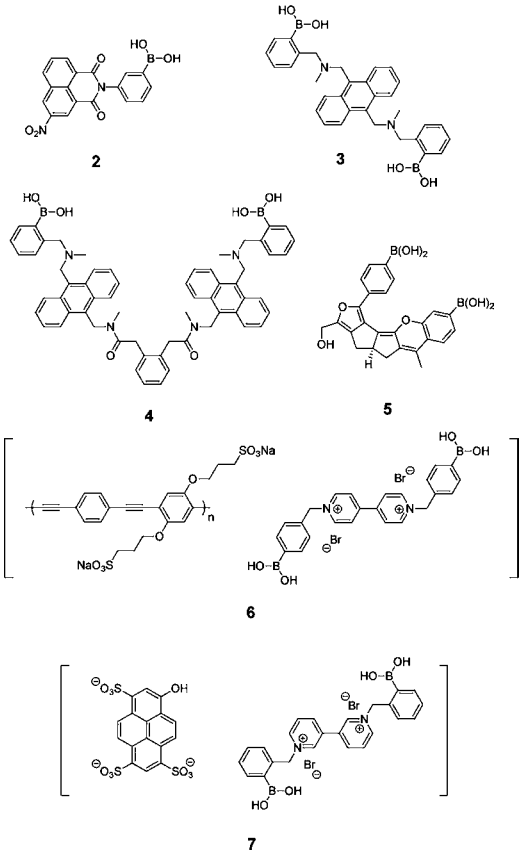

regime which is the most modulating, sensitive, and selective sensor in its class to-date.

■ ASSOCIATED CONTENT

Supporting Information

Complete experimental details for all new compounds and absorption and fluorescence data for **1** with saccharides (D-glucose, D-fructose, D-galactose, and D-mannose) and nucleotides (guanosine, adenosine, uridine, and cytidine). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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