Sensor Arrays

DOI: 10.1002/ange.200701888

# A Fluorescent Sensor Array for Saccharides Based on Boronic Acid Appended Bipyridinium Salts**

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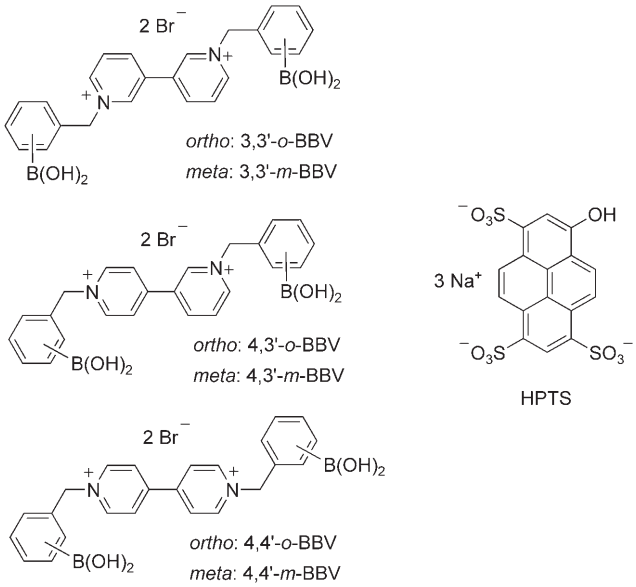
The detection of small biomolecules is of central interest in medical diagnostics.[1] Inspired by the cross-responsive strategy of the mammalian olfactory system,[2] several impressive examples of sensor arrays[3] for small bioanalytes such as amino acids,[4] peptides,[5] proteins,[6] nucleic acids,[7] steroids,[8] and carbohydrates[9] have been reported. In some of these sensor arrays, indicator displacement assays (IDAs)[10] were used. The modular sensing ensemble of an IDA with its variable indicator/receptor/analyte ratio[11] is ideally suited for implementation in sensor arrays. Recognition patterns ("fingerprints") of analytes can be easily measured by varying not only the receptor and the indicator but also by choosing different sensing conditions, such as the pH value or solvent medium.[5,9a]

Over the last five years, our group has developed a saccharide-sensing system similar to an IDA that comprises boronic acid receptors and fluorescent dyes.[12] Herein, we describe the use of designed receptor units in an IDA-based sensor array to differentiate saccharides in aqueous solution at neutral pH values. Chang and co-workers recently published a study of colorimetric discrimination of 23 carbohydrates at 100 mM concentration based on a pH-indicator/pH-change-inducer array.[9b] We are able to discriminate among twelve saccharides at a concentration of 2 mM by using a fluorescent sensor array at physiological pH values.

The modular sensing ensemble is composed of the anionic fluorescent dye, 8-hydroxypyrene-1,3,6-trisulfonic acid trisodium salt (HPTS), and an array of six cationic bis-boronic acid appended benzyl viologens (BBVs, Scheme 1). We reported that synthetically varying the spacing between the diboronic acids provided enhanced glucose selectivity.[12a]

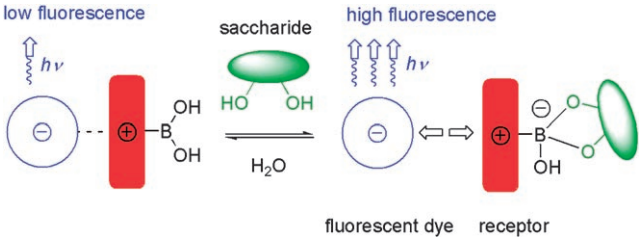
Ground-state complex formation[13] between the anionic fluorescent dye and a cationic BBV receptor facilitates electron transfer from the dye to the viologen, resulting in a decrease in the fluorescence intensity of the dye




***Scheme 1.*** Saccharide sensing ensemble of an array of six cationic bis-boronic acid appended benzyl viologens and the anionic fluorescent dye, 8-hydroxypyrene-1,3,6-trisulfonic acid trisodium salt (HPTS).

(Scheme 2).[14] When a saccharide is added to the ground-state complex, the boronic acids are converted into anionic boronate esters, partially neutralizing the net charge of the cationic viologen.[15] This reduces the quenching efficacy and increases the fluorescence intensity.

The binding characteristics of all six BBV receptors with HPTS have been reported in the form of Stern–Volmer (S-V) quenching constants.[12a] It was shown that S-V constants correlate with the association constants that were determined from UV/Vis absorbance data.[12d,e] The static quenching constants varied from $4.3\times10^3$ to $8.9\times10^3\,\text{M}^{-1}$. The binding constants of selected saccharides such as D-glucose, D-fructose, and D-galactose with the BBV receptors are one or two magnitudes lower.[12a] The competitive conditions, which are necessary for an IDA to work, were obtained by choosing




***Scheme 2.*** Proposed saccharide-sensing mechanism based on a BBV receptor and the anionic fluorescent dye HPTS.

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[**] We thank GluMetrics, Inc. and the UC BioStar Industry-University Cooperative Research program (grant bio04-10458) for financial support. We thank Dr. S. Gamsey, L. Hirayama, B. Vilozny, and J. Gardina for the synthesis of the BBVs and the ETOX Laboratory (UCSC) for providing the fluorescence plate reader. We thank A. Buryak (École Polytechnique Fédérale de Lausanne, EPFL) for helpful discussions on the LDA.

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

a ratio of 1:125:500 for the sensing ensemble HPTS/BBV/saccharide.

For the discrimination study, the following twelve saccharides were chosen: D-ribose, D-arabinose, L-rhamnose, D-xylose, D-lyxose, D-glucose, D-mannose, D-galactose, D-fructose, L-sorbose, melibiose, and lactulose. All saccharides possess free hydroxy groups in the 1- and 2-positions, allowing them to bind to the boronic acid.[16] The increase in fluorescence intensity $F/F_0$ after adding the saccharide to the BBV/HPTS sensing ensemble was measured with a fluorescence plate reader (see the Supporting Information).

Among five selected saccharides (D-ribose, D-glucose, D-fructose, melibiose, and lactulose; Figure 1), the ketoses, D-fructose and lactulose, showed the highest response of all six BBV receptors. Typically, *ortho*-BBVs exhibited greater $F/F_0$ values than *meta*-BBVs. The superior performance of *ortho*-BBVs has been attributed to the intramolecular electrostatic interaction between the anionic boronate ester and the positively charged nitrogen of the viologen.[12b]

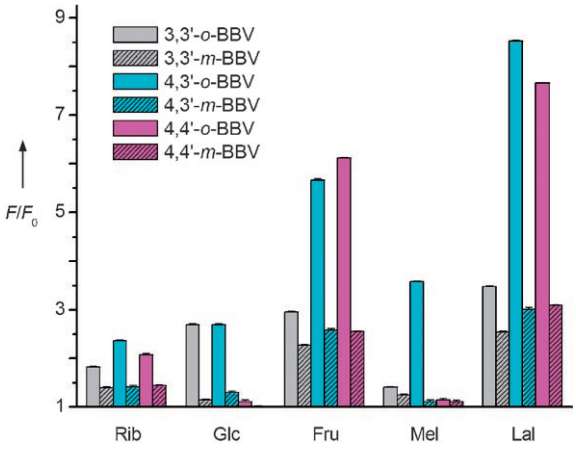


***Figure 1.*** Relative fluorescence increase ($F/F_0$) of HPTS ($4.0\times10^{-6}$ M) with all six BBV receptors (3,3′-*o*-, 3,3′-*m*-, 4,3′-*o*-, 4,3′-*m*-, 4,4′-*o*-, and 4,4′-*m*-BBV; $5.0\times10^{-4}$ M) after adding saccharides (D-ribose (Rib), D-glucose (Glc), D-fructose (Fru), melibiose (Mel), and lactulose (Lal)) at a final concentration of $2.0\times10^{-3}$ M (phosphate buffer solution, pH 7.4, 39 mM). Errors, given in a 95.5% confidence interval, are lower than 2.3%.

The statistics program SYSTAT[17] was used to perform a linear discriminant analysis (LDA)[18] of the $F/F_0$ data. Only "jackknifed" classification matrices[19] were taken to evaluate discrimination results. When using all six BBV receptors, 100% accurate discrimination was achieved for all twelve saccharides. The *F* value of each receptor, which indicates the relative importance in the LDA, showed that the contribution of the 3,3′-*m*-, 4,3′-*m*-, and 4,4′-*m*-BBV was minor compared with the corresponding *ortho* derivatives, 3,3′-*o*-, 4,3′-*o*- and 4,4′-*o*-BBV (see the Supporting Information). Apparently, three receptors are sufficient to fully discriminate the set of saccharides. This was confirmed by a 100% accurate discrimination when using only 3,3′-*o*-, 4,3′-*o*-, and 4,4′-*o*-BBV in an LDA. The results are depicted in a graphical score plot (Figure 2). The data of all twelve saccharides appear visually distinguishable. To some extent, chemical and structural

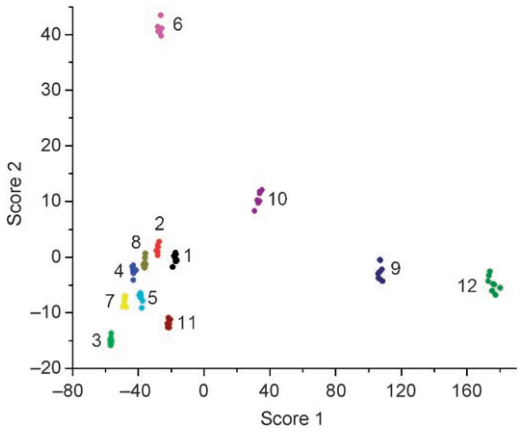


***Figure 2.*** Two-dimensional LDA score plot for the identification of twelve saccharides by using three receptors: 3,3′-*o*-, 4,3′-*o*-, and 4,4′-*o*-BBV. Saccharides ($2.0\times10^{-3}$ M): D-ribose (1), D-arabinose (2), L-rhamnose (3), D-xylose (4), D-lyxose (5), D-glucose (6), D-mannose (7), D-galactose (8), D-fructose (9), L-sorbose (10), melibiose (11), lactulose (12).

similarities of the saccharides were reflected by the relative position of their clustered data. The scores of the aldoses are positioned in proximity to each other. In contrast, the data of D-glucose and the ketoses D-fructose, L-sorbose, and lactulose are well separated from the others because ketoses generally exhibit higher binding affinities to boronic acids than aldoses. The extraordinary behavior of D-glucose can be explained by the exceptional contribution of 3,3′-*o*-BBV and 4,3′-*o*-BBV in the LDA (Figure 1). To verify that the discrimination between the analytes did not arise from small differences in the concentrations of the saccharide stock solutions, we reduced the concentration by 5%. Thus, $F/F_0$ data of 3,3′-*o*-, 4,3′-*o*-, and 4,4′-*o*-BBV with saccharide concentrations of 1.9 mM and 2.0 mM were used to perform an LDA; there was still 100% accurate discrimination.

To evaluate the contribution of each BBV receptor to the discriminatory power in the LDA, we combined all six BBV receptors into groups with three members. The resulting twenty distinct combinations were used in an LDA for the discrimination of our set of twelve saccharides. Ten of the twenty combinations containing at least two *ortho*-BBV receptors were able to achieve 100% accurate discrimination. In contrast, not all of the saccharides were correctly differentiated by the ten other combinations, which contain at least two *meta*-BBV receptors. The conclusion is that *ortho*-BBV receptors have greater discriminatory power than *meta*-BBV receptors. From the standpoint of a sensor array design, the use of *ortho*-BBV receptors is thus the most desirable.

The results described herein demonstrate the analytical power of an array of BBV receptors to differentiate among twelve closely related saccharides at 2 mM concentration in aqueous solution. *ortho*- and *meta*-BBV receptors displayed different discriminating power.

Received: April 29, 2007
Revised: June 20, 2007
Published online: July 25, 2007

  

**Keywords:** boronic acids · carbohydrates · fluorescence · sensors · viologens


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