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The Development of Boronic Acids as Sensors and Separation Tools

JOHN S. FOSSEY,[1]* FRANCOIS D'HOOGE,[1] JEAN M. H. VAN DEN ELSEN,[2] MARTA P. PEREIRA MORAIS,[2] SOFIA I. PASCU,[3] STEVEN D. BULL,[3] FRANK MARKEN,[3] A. TOBY A. JENKINS,[3] YUN-BAO JIANG,[4] AND TONY D. JAMES[3]

[1]The School of Chemistry, The University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK.
[2]University of Bath, Department of Biology and Biochemistry, Bath, BA2 7AY, UK.
[3]University of Bath, Department of Chemistry, Claverton Down, Bath, BA2 7AY, UK.
[4]Department of Chemistry, College of Chemistry and Chemical Engineering, and the MOE Key Laboratory of Analytical Sciences, Xiamen University, Xiamen 361005, China.
E-mail: j.s.fossey@bham.ac.uk

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ABSTRACT: Synthetic receptors for diols that incorporate boronic acid motifs have been developed as new sensors and separation tools. Utilizing the reversible interactions of diols with boronic acids to form boronic esters under new binding regimes has provided new hydrogel constructs that have found use as dye-displacement sensors and electrophoretic separation tools; similarly, molecular boronic-acid-containing chemosensors were constructed that offer applications in the sensing of diols. This review provides a somewhat-personal perspective of developments in boronic-acid-mediated sensing and separation, placed in the context of the seminal works of others in the area, as well as offering a concise summary of the contributions of the co-authors in the area.
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Introduction

Diols reversibly interact with boronic acids to form boronic esters in aqueous media,[1] whilst it has been known that boric acid is important in the determination of saccharide configurations for almost 100 years.[2] It was 50 years later that the corresponding interactions of diols with boronic acids were first reported.[3] Reversible boron-diol interactions, under basic conditions, establishes an equilibrium between the diol-unbound boronate and the diol-bound boronate ester (Figure 1). In earlier work, the general areas of boronic-acid-mediated sensing[4] and self-assembly[5] that exploit boron-diol interactions have been broadly addressed. Herein, a personal perspective is presented that provides an in-depth discussion pertaining to the contributions of the co-authors in the area of boronic-acid-mediated sensing and separation, along with reference to the salient works of others in this fast-moving field.

Sensors

Molecular sensors, or chemosensors, report a molecular interaction through some kind of readable output. Molecular sensors can be found in various aqueous biological systems. The ability of nature's sensors to expel water from binding sites to requisition analytes in a non-covalent fashion is an extraordinary achievement. In the saccharide-recognition arena, unimolecular synthetic receptors that only use non-covalent interactions to bind low concentrations of saccharide analytes in competition with solvent water molecules are not well-reported. However, Davis and co-workers have developed exquisite hydrogen-bonding receptors for saccharides that are capable of binding glucose in water.[6] In 1992, Yoon and Czarnik detailed an anthracene boronic acid that was capable of fluorescently signaling the presence of polyols.[7] James, Shinkai, and co-workers followed this work up with boronic-acid-based glucose-selective and asymmetric saccharide-recognition sensors.[8] The well-established relative binding constants (K) of monosaccharides with boronic acids reveals glucose to be among the weaker boronic acid binders,[1a] which presents a problem in the development of glucose selectivity as a desirable trait in the design of artificial receptors. The incorporation of a judiciously positioned second boronic acid motif within the molecular-chemosensor construct offered glucose-selectivity options.[9]

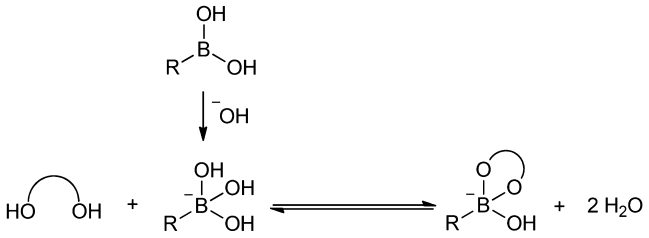


Fig. 1. Equilibrium that shows diol binding to boronate.

It is important to remember that synthetic chemosensors may often be required to function in aqueous environments. Critical for such sensors is a nearby nitrogen atom that offers the potential for a boron-nitrogen interaction. Not only is such an interaction a fundamental part of the signaling mechanisms but it also helps the sensors to function in aqueous media. Anslyn and co-workers have studied the interaction of appended amines with boronic acids and esters[10] and they reported the potential importance of solvent molecules in boron-nitrogen interactions. Computational studies have the potential to reveal the interaction modes of water in molecular systems[11] and in boronic-acid-based sensors.[10] More recently, Larkin et al. identified one plausible H-bonding interaction,[12] which was followed by Mulla et al. who identified a similarly incorporated water molecule (Figure 2).[13]

Click-Fluor

"Click-fluor" is a term that we have used to describe a fluorescent molecule that exhibits a new fluorescence band as a consequence of its construction through a Huisgen [3 + 2] cycloaddition.[14] The so-called "click reaction"[15] delivers 1,2,3-triazole rings from azides and terminal alkyne starting

John S. Fossey (principal author, see photo) is a Lecturer in chemistry at the University of Birmingham. Prior to this position, he held a temporary position at the University of Bath where an interest in boronic acids was cultivated. **Francois D'Hooge** is a Research Scientist at Spirogen (UK). He has previously spent postdoctoral stints at the Universities of Bath and Birmingham where he worked on boronic-acid-based sensors. **Jean M. H. van den Elsen** is a Reader in biology and biochemistry at the University of Bath. He is interested in the development of boronic-acid-containing gels as separation tools for carbohydrates and carbohydrate-modified proteins. **Marta P. Pereira Morais** performed her undergraduate project in chemistry at the University of Bath, where she obtained a BSc in natural sciences. She obtained a PhD in biology and biochemistry at the same institution in related boro-gel topics. **Sofia I. Pascu** is a Royal Society University Research Fellow and Lecturer in inorganic chemistry at the University of Bath. She is interested in the use of boronic-acid-functionalized probes in molecular-imaging applications. **Steven D. Bull** is a Reader in organic chemistry at the University of Bath. He was instrumental in the development of enantiodiscrimination/determination by using boronic acids. **Frank Marken** is a Professor in physical chemistry at the University of Bath. His expertise in electrochemistry has been applied to the development of electrochemically reporting boronic-acid-based sensors. **A. Toby A. Jenkins** is a Reader in physical chemistry at the University of Bath. He is interested in the use of surface- or interface-bound boronic acids in new signaling regimes, especially through the use of surface plasmon resonance. **Yun-Bao Jiang** is the Minjiang Chair Professor of chemistry at the Department of Chemistry, Xiamen University. He has developed a variety of new sensing procedures by using boronic acids and has extensively collaborated with researchers at the Universities of Bath and Birmingham. **Tony D. James** is a Professor of organic chemistry at the University of Bath. He is the founding member of the strong interdisciplinary cohort of scientists that are working on boronic acid chemistry in, and from, Bath.

materials. By preparing a boronic acid azide in situ and exposing it to a terminal alkyne under common "click" conditions, the molecule shown in Figure 3 was prepared.

The "click-fluor" concept is versatile, not only because a new fluorophore is generated upon triazole formation,[16] but also owing to the wide availability of acetylene units that facilitate diversity in the sensor architecture. As such, it is ideally suited to modular synthetic approaches[17] and the triazole motif has been incorporated into a number of related systems that have, or may be, applied to sensing.[18] The same original click-fluor motif was employed by White et al. in a study of the reactions of the boronic acid fragment;[19] this concept was applied in the sensing arena by Xu et al., where the rate of a palladium catalyzed cross-coupling reaction that delivered a probe molecule was modulated by the presence of sugars, thus acting as an indirect, but very effective, probe for saccharides.[18b] The application of polyalkynes, which are in themselves versatile building blocks in organometallic chemistry[20] and have the potential to be developed into bespoke, selective, and complex click-fluor sensors, was elegantly demonstrated by Shao and Zhao.[18c]

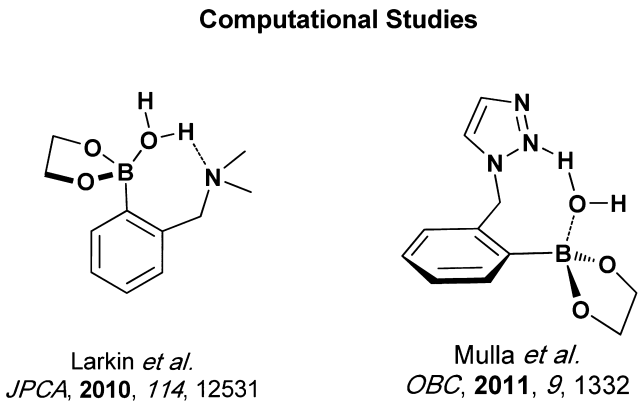


Fig. 2. The boron-nitrogen interaction was computationally revealed to result from a bridging water molecule in aqueous media.

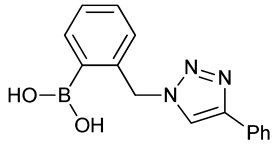


Fig. 3. The first "click-fluor".

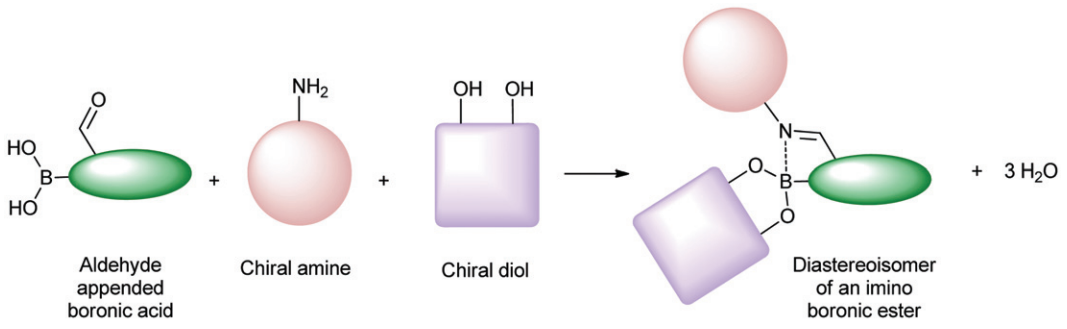
Determination of Enantiomeric Excess

Bull, James, and co-workers have published a series of papers that detail the use of a three-component reaction between an aldehyde-appended boronic acid, a chiral diol, and a chiral amine. Such constructs are used to probe the enantiomeric excess of either the chiral diol or the chiral amine by measurement of the ratio of the resultant diastereoisomers by NMR spectroscopy when a single enantiomer of the partner amine or diol is used to complete the triplicate set in the three-component reaction (Scheme 1).[21] A similar three-component reaction was also exploited in the synthesis of bis-boron self-assembled architectures with applications in the supramolecular chemistry arena, wherein reversible self-assembly was demonstrated.[22]

The same technology has also been extended to the development of electrochemically responsive systems through the incorporation of a ferrocene derivative, thereby allowing for the electrochemical determination of enantiomeric excess.[23] Electrochemical sensors for saccharides[24] and α-hydroxy acids have also been developed.[25]

Fluorophore-Quencher Conjugates

Surface plasmon resonance (SPR) is a phenomenon that is used for measuring binding interactions at surfaces (commonly, a gold surface is employed). Through first attaching specific receptors onto a surface, the binding of analytes to that receptor can be assessed by SPR. As such, SPR finds routine application in probing biological recognition events. Thus,



Scheme 1. Three-component reaction for the boron-mediated determination of enantiomeric excess.

confining binding events to those on or nearby a planar gold surface can allow much data regarding the analytes and their binding to be gained. To apply this well-established technique to boronic-acid-diol recognition, we chose to modify a gold-streptavidin self-assembled monolayer system.[26] In addition to the regular SPR signals, we wanted to incorporate the possibility of using surface plasmon excitation to excite incorporated fluorophores, thus offering a dual SPR–fluorescence-detection regime. This regime has a significant advantage in fluorescence detection over more-traditional excitation-emission ensembles because no incident light may enter the system. Thus, any observed light that is detected is only due to emission from the fluorophores near by the surface (owing to surface plasmon excitation effects). To take advantage of existing gold-streptavidin technology, a biotin-fluorophore-boronic-acid conjugate was prepared (coined FLAB: Fluorophore Linker boronic Acid Biotin, Figure 4). We had the idea that, with FLAB in hand, we could construct a boronic-acid-appended surface that would respond to quencher diols (as shown previously in solution[27]) and give a concomitant SPR signal (Figure 5).

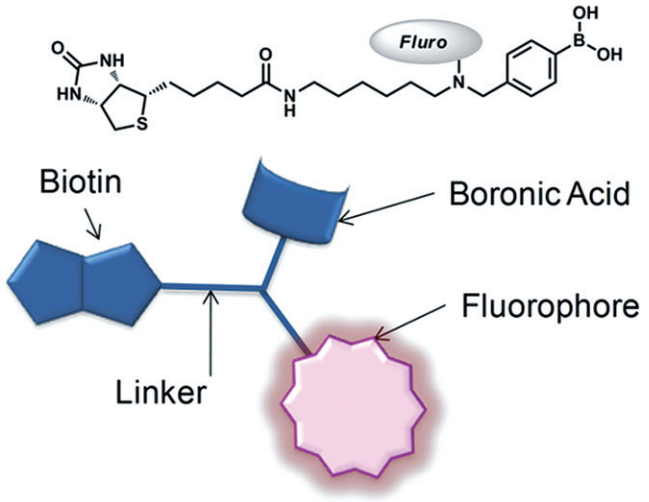


Fig. 4. FLAB–fluorophore linker boronic acid biotin.

Fluorophore excitation at a gold surface by surface exciton requires good matching of the fluorophore-excitation properties with the surface plasmon; in other words, an energy gradient is required to transfer energy from a surface to a nearby fluorophore, which means that correct fluorophore selection is crucial. For planar gold, a good fluorophore is the commercially available Alexafluor 647; however, cost limits its synthetic utility, so we constructed an Alexafluor-FLAB conjugate alongside a Fluorescein-FLAB counterpart on a larger scale by using the same FLAB precursor as a control (Scheme 2).[26]

The binding of FLAB-Alexafluor by diol-appended BHQ was confirmed to be a one-to-one interaction in solution[28] and FLAB-Alexafluor was shown to bind to the surface-appended streptavidin by SPR and fluorescence spectroscopy.[26] Indeed, the addition of BHQ-diol to the streptavidin-FLAB-appended

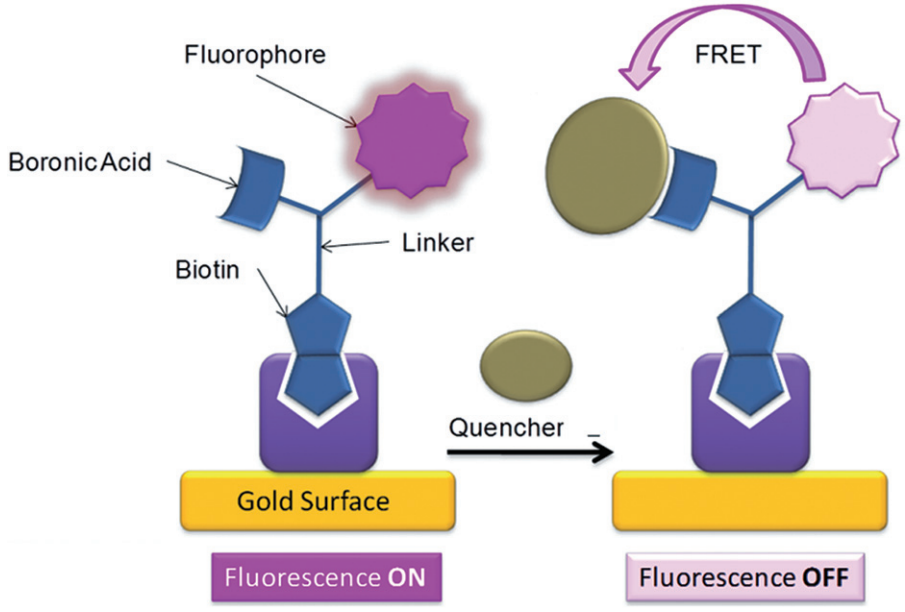
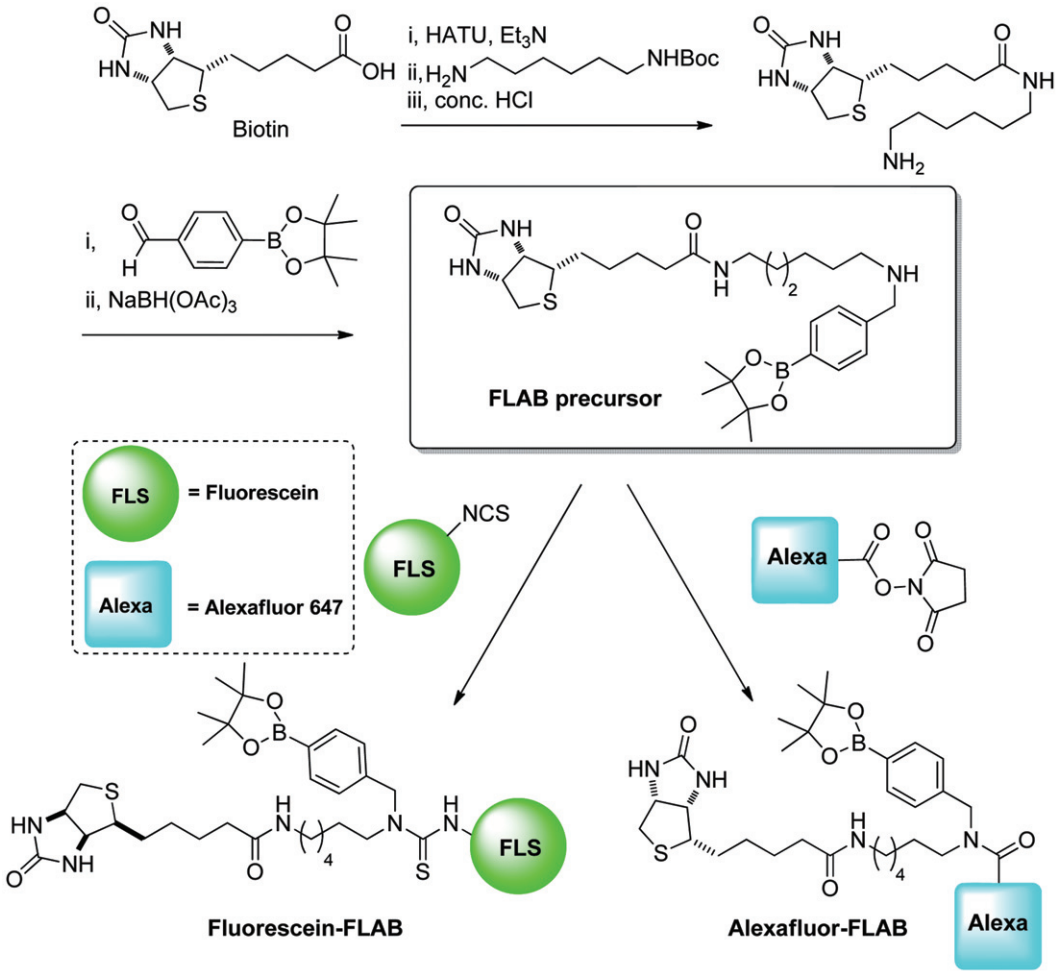


Fig. 5. Surface-appended FLAB (fluorophore linker boronic acid biotin) for use as a fluorescence surface plasmon resonance (f-SPR) sensor.



Scheme 2. Preparation of FLAB-Fluorescein and FLAB-Alexafluor from the same precursor.

gold surface revealed a dual-mode sensing event, whereby an increase in the SPR signal was accompanied by a decrease in fluorescence, thus demonstrating a model sensing regime for quencher diol detection. The two quencher-appended diols used are shown in Figure 6.

Taking advantage of biotin-appended sensors in other work led us to investigate another model system that included microscale avidin-appended polystyrene microspheres (Bang Labs, Figure 7). We were able to functionalize microspheres (10 μm size) with FLAB fluorescein, as demonstrated by epifluorescence fluorescence microscopy (λ_{ex} = 450–490 nm).[28]

With fluorescent boronic-acid-quencher conjugates in hand, we envisaged a quencher-elimination strategy for the detection of diols, such as saccharides, whereby a quenched conjugate could recover fluorescence (fluorescence on) through exchanging the bound quencher for a diol analyte (Scheme 3). After testing the quencher-elimination strategy in various competition experiments in solution with FLAB, we demonstrated its suitability in imaging applications by exposing the FLAB-Fluorescein-microsphere conjugates to a methyl-red-inspired diol-appended quencher (a quencher-appended diol with a structure that is similar to methyl red, Figure 6); as expected, the images showed a loss of fluorescence. Gratifyingly, upon addition of fructose, the fluorescence was recovered. Figure 8 shows the FLAB-Fluorescein-microsphere conjugates, the quenched scenario, and the final fluorescence-recovered situation after exposure to fructose.[28]

Dye Displacement Assay

A dye-displacement sensor is a colorimetric competitive-binding assay that uses an analyte to displace a dye from a receptor, wherein the displacement manifests itself as a color change that is related to the presence of an analyte. Key players

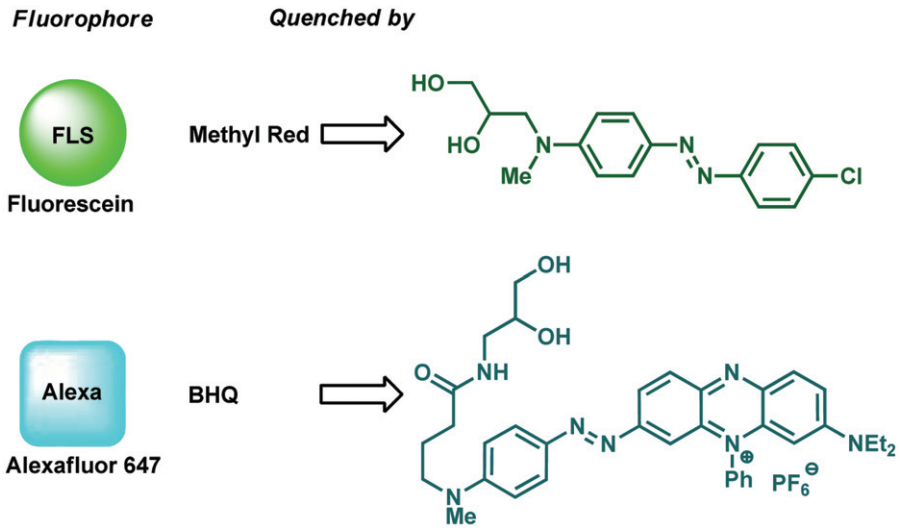


Fig. 6. Diol-appended quenchers with matched excitation profiles for fluorescein (upper) and Alexafluor647 (lower); BHQ = black hole quencher.

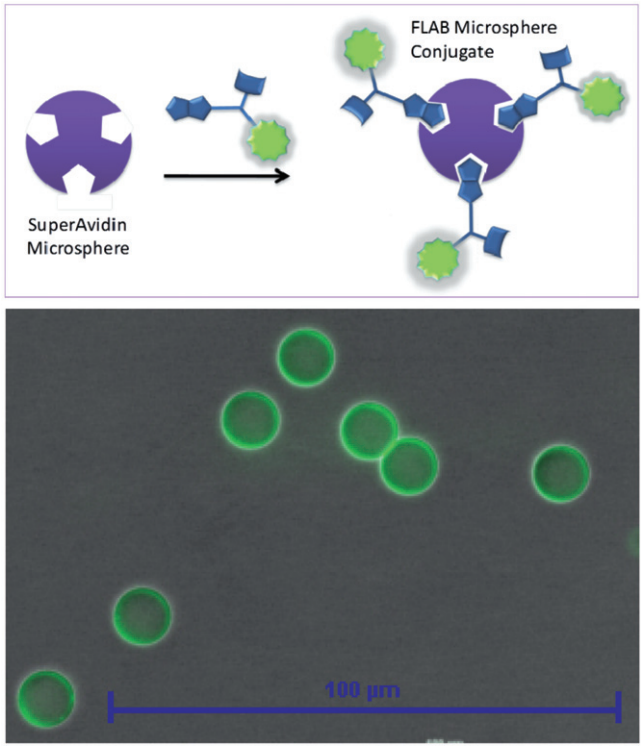
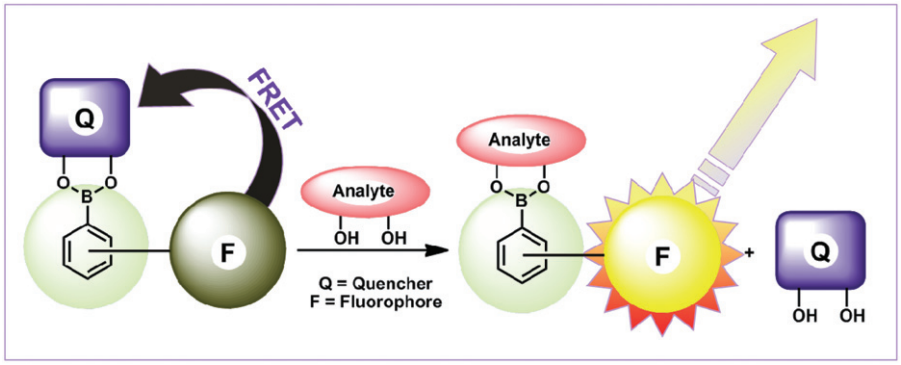


Fig. 7. Top: Schematic of the functionalization of SuperAvidin microspheres with fluorescein-FLAB. Bottom: Overlaid fluorescence and bright-field images of FLAB-functionalized microspheres.

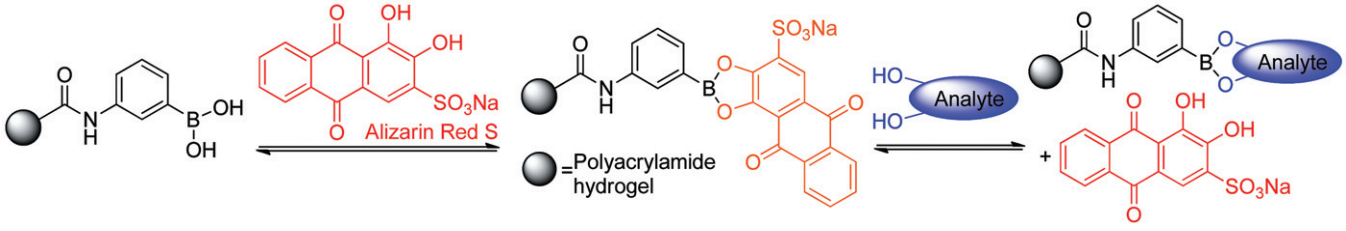
in the field of supramolecular sensing and the use of dye-displacement assays include the groups of Anslyn,[29] Severin,[30] and Singaram.[31] Dye-displacement assays offer several advantages over other assay formats, including the fact that the receptor does not require the receptor to be covalently bound to the dye or to the indicator and that parallel dyes may be displaced in the same assay,[29g] thereby offering multiplexing opportunities.

For boronic acids, alizarin red-S (ARS) is a good dye for use in such competitive-binding assays because the catechol motif not only provides a reversible binding handle but it also results in a hypsochromic shift that indicates ARS binding to boron, that is, ARS turns orange on binding to boron.[32] Boron-containing polymers have attracted interest in electronic and sensing applications[33] and hydrogels have also been used in dye-displacement assays.[34] Kim et al. have described how the solubility of boronic-acid-containing polymers may be modulated by the presence of saccharides.[35] Phenylboronic acid has also been used in combination with ARS in a study in which thermally responsive glucose-appended polymers influenced bacterial aggregation.[36] Very recently, polymers that incorporated boronic acids have been used to elegantly release insulin in response to a lowering of the glucose concentration.[37] In our contribution, we produced hydrogel spheres (5 mm in diameter) that incorporated phenylboronic-acid units that were exposed to ARS dye, thereby forming their corresponding boronic ester. After washing, exposure to fructose released the dye into solution (Scheme 4), allowing the relative amounts of boron-binding species (saccharides) in samples of fruit juices to be determined.[38]

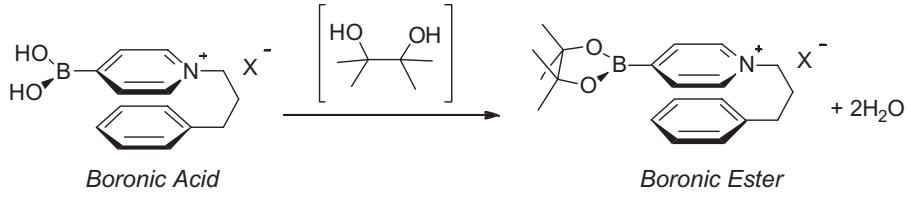
Slabs of gel with and without boron were prepared and exposed to ARS (Figure 9, a boron-containing gel prior to exposure to ARS is also shown for comparison); the orange color of the boron-containing sample versus the red color of the non-boron-containing sample is indicative of a boron-ARS



Scheme 3. Quencher elimination strategy for sensing diols.



Scheme 4. Binding and analyte-provoked release of alizarin red-S with a hydrogel-bound boronic acid.[38]



Scheme 5. Phenyl propylpyridium boronic acid as a receptor for diols.

interaction. Hajizadeh et al. independently reported a similar system around the same time,[39] where an interaction with glucose was studied.

Cation-π Interactions

Studies by Bull, Fossey, and co-workers revealed that pyridinium-cation-π interactions may be investigated by fluorescence,[40] whilst it has also been suggested that such interactions may be important in organocatalysis.[41] Because we had already developed a pyridinium-boronic-acid sensor for saccharides,[25] it seemed logical to combine cation-π interactions and pyridinium boronic acids into one construct for diol detection. In our preliminary findings, we were able to show that a simple propylene (Leonard linker)[42]-linked phenyl alkyl pyridinium boronic acid showed characteristic cation-π-stacking exciplex fluorescence.[43] Of the anions that were probed, we showed that the most-intense fluorescence response came from boronic-acid-bearing complexes with the most-diffuse negative charge ($X^- = PF_6$, Scheme 5). The relative fluorescence intensity ($PF_6^- > Br^- > Cl^- > F^-$) may be interpreted as the tighter the ion pair the weaker the fluorescence; thus, for a "turn-on" sensor, it is better to start with a low-fluorescence (tight-pair) situation that has the potential to undergo fluorescence enhancement upon interaction with an analyte.

Indeed, for the sensing regime depicted in Scheme 5, when $X = PF_6$, no fluorescence change was observed; essentially, the starting position was already at maximum fluorescence potential. However, the best "fluorescence on" response was observed for X = Cl (Figure 10). The precise nature of the role of boron hybridization and the extent of the overlap of its empty orbital with the electron-deficient pyridinium, as well as the consequences for π stacking, are currently being investigated by us.

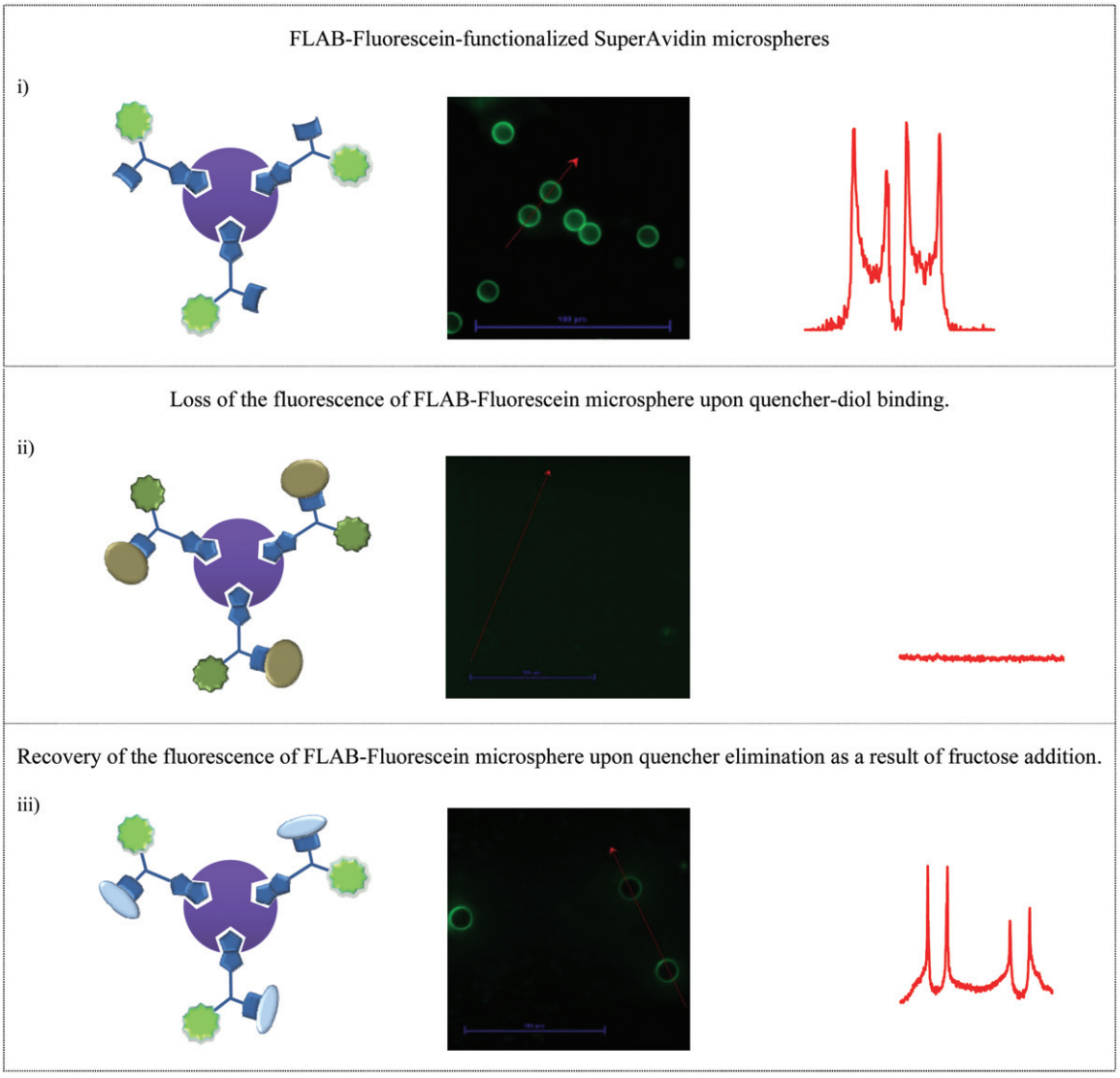


Fig. 8. Left: Schematic of FLAB-Fluorescein-functionalized SuperAvidin microspheres. Centre: Fluorescence images. Right: y = Brightness of the image along the 1D track (red arrow); x = number of pixels. i) Start situation: FLAB-Fluorescein-treated microspheres and the tracking fluorescence plot across the spheres. ii) Quencher added: FLAB-Fluorescein-bound microspheres after exposure to a quencher diol and the tracking fluorescence plot across the spheres. iii) Fructose added: Increase in fluorescence after quencher displacement that results from the addition of fructose to FLAB-quencher-conjugate-appended microspheres and the tracking fluorescence plot across the spheres.

Electrochemical Assays

Boronic-acid functionality has been employed in electrochemical sensing systems that were predominantly based on the direct effects of analyte binding on current and/or potential responses in voltammetric experiments. In a recent review, the role of phenylboronic acids, in particular for electrochemical sugar sensing, was highlighted.[44] Broadly speaking, electrochemical assays that employ boronic acids can be divided into solution-phase processes and surface-immobilized processes.

Solution-Phase Processes

The most-widely studied probes are soluble ferrocenylboronic acid redox probes, which have been shown to allow the direct electrochemical sensing of saccharides in aqueous media.[45] The reversible binding of saccharides was studied as a function of pH value[46] and for various diols.[47] The potential for the environmental analysis of diols and phenolic compounds has been proposed.[48] Mono- and diferrocenyl complexes have been characterized and studied[49] and improved ferrocenylbo-

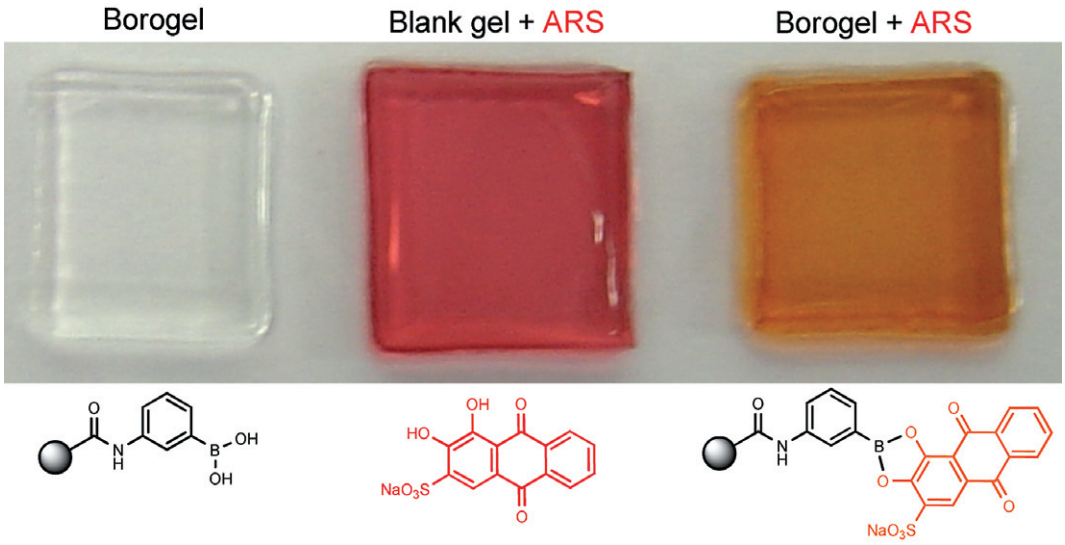


Fig. 9. Slabs of gel: Borogel (left); blank gel + alizarin red-S (middle) and borogel + alizarin red-S (right).[38]

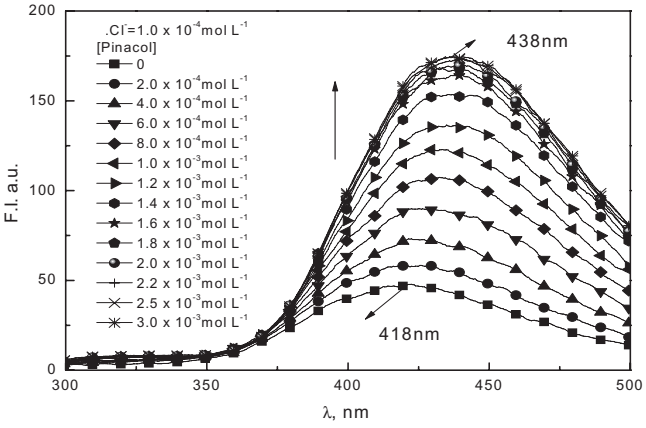


Fig. 10. Fluorescence enhancement as a result of increasing pinacol concentration (according to Scheme 5).

ronic acids have been proposed for fluoride,[50] glucose, and saccharide sensing.[51] Chiral ferrocenylboronic acids have been reported for chiral electroanalysis.[23] Boronic acid moieties that are linked to other redox probes, such as Fe-bipyridyl complexes,[52] phenazines,[53] or tetrathiafulvalenes,[18c] have been reported. In some cases, the analyte itself can act as the redox probe, for example, for catechols when bound to phenylboronic acid.[54]

Surface-Immobilized Processes

Owing to electrochemical processes fundamentally being heterogeneous in nature, much-more-sensitive and, probably, more-selective sensing processes can occur directly at the electrode/solution interface. A considerable number of studies have been devoted to conducting polymer systems, in particular polypyrrole[55] and polyaniline[56] films that contain covalently bound phenylboronic acid. These polymer-sensing films are conveniently prepared by electropolymerization[57] and the polymer matrix can function to further increase selectivity.[58]

The binding of ferrocenylboronic acid to surface-immobilized hemoglobin protein was demonstrated as a method to indicate protein-glycation levels in blood with an amperometric biosensor.[59] Nanostructuring has been applied to improve sensitivity, for example, by surfactant templating to give nanofibers of polyaniline boronic acid [60] or by controlled co-polymerization.[61] Multiwalled carbon nanotubes that were functionalized with boronic acids[62] or formed into nanostructured composites[63] have been shown to exhibit high sensitivity to *ortho*-quinols, such as dopamine. Carbon nanotubes that were surface-functionalized with 3-aminophenylboronic acid have been employed to "capture" leukemia cells and as a reusable cyto-sensor.[64]

Monolayer films of boronic acids at electrode surfaces have been developed based on diazonium grafting onto glassy carbon [65] and based on self-assembled monolayer (SAM) methods on gold.[66] Amphiphilic *N*-hexadecyl-pyridinium-4-boronic acid cations have been self-assembled into a monolayer at graphite electrodes.[66d] Self-assembled monolayers based on 3-amino-phenylboronic acid on gold have been exploited in immunosensors[67] in which the boronic acid plays predominantly a structural role.

Finally, in recent studies on biphasic redox systems, microdroplet deposits of water-insoluble organic liquids at electrode surfaces have been employed with dissolved boronic acids. In

this unique system, highly hydrophobic naphthyl- and anthracenyl derivatives of boronic acids are dissolved in a 4-(3-phenylpropyl)-pyridine solvent. An additional redox system, such as tetraphenylporphyrinato-manganese(II/III), allows anions to be actively transferred from the aqueous to the organic phase with selectivity for anions with an affinity for boronic acids. The transfer of carbonate and bicarbonate,[68] as well as the transfer of α-hydroxy-carboxylates, have been reported.[25] These biphasic redox systems could also be incorporated into hydrophobic membranes for separation purposes.

Separation

Affinity chromatography exploits reversible or non-covalent interactions at a stationary separation domain to enhance or modulate the separation of analytes. The reversible interaction of boron with diols, as described earlier, has been exploited in the separation of diol-containing analytes through the incorporation of boronic acids into chromatographic stationary phases,[69] especially in the boron-affinity columns that are used for HPLC.[70]

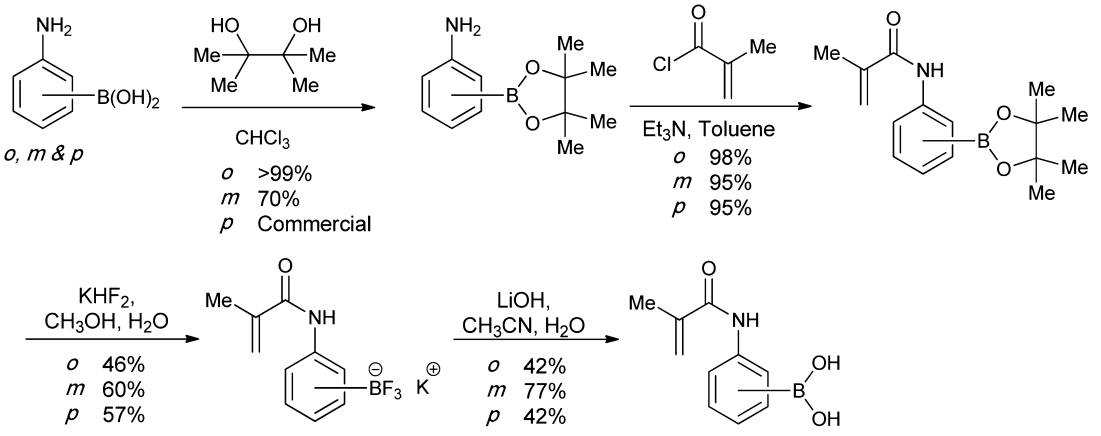
BASE

In our contribution, a polyacrylamide-based gel-electrophoresis technique, which had already been used for carbohydrate analysis (called FACE, fluorophore-assisted carbohydrate electrophoresis), was modified by the incorporation of boronic acids into the gel domain. FACE is a good technique for separating saccharides on a size and charge basis,[71] but it has some drawbacks because labeling is required, whilst saccharides of similar size and charge are not well resolved. Glycoconjugates of proteins may be visualized by staining, but differentiation between glycated and glycosylated proteins of otherwise similar size and charge is difficult.

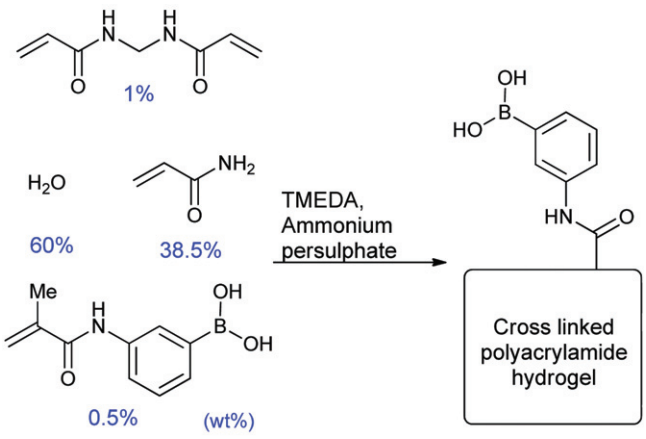
We developed boron affinity saccharide electrophoresis (BASE)[72] to address the need for a swift, reliable, and easy-to-adopt electrophoresis affinity technique. Because different saccharides have different affinities for boronic acids, multiple mobility-modulating interactions can take place during gel transit; these interactions have a cumulative effect that can be used to separate saccharides (and conjugates thereof) with similar size and charge on the basis of their boron affinity. Thus, we set about devising a procedure for the incorporation of boronic acid motifs into hydrogels. Previously reported boronic-acid-based sensors were inspirational in their design[73] and a range of acrylamide boronic acids were synthesized[74] and easily incorporated into polyacrylamide hydrogels for electrophoresis.[72]

A two-step deprotection strategy for the synthesis of acrylamido boronic acids (D'Hooge's acrylamide-boronate deprotection was developed by following the methodology reported by Molander and Ellis[75]) was critical in obtaining anything other than poor yields of *ortho*-, *meta*-, and *para*-methacrylamido phenylboronic acids (Scheme 6). In our hands, direct pinacol deprotection led to unwanted polymerization as a significant side-reaction. Polymerization was sometimes problematic, but avoiding unnecessary manipulations and heating during the isolation and purification stages helped to improve the yield of the isolated product.[74] Later, it was confirmed that, in the polymer-gel-phase experiments, the deprotection of pinacol occurred in situ; thus, pinacol- or, preferably, glycol-protected boronic acid acrylamides may be used directly, thereby offering a significant advantage in terms of yield.

For our comparison of boron affinity (BASE) versus the FACE technique, an early optimal formulation for polymer formation was found to consist of 60 wt% water, 0.5 wt%



Scheme 6. Synthesis of methacrylamido boronic acids.



Scheme 7. Gel formulation.

boron-containing monomer, 1 wt% methylene bis-acrylamide (cross-linker), and 38.5% acrylamide (Scheme 7). Non-boron-containing control gels were prepared with 0.5% phenylmethacrylamide (in place of the boronic acid), whilst blank gels were prepared by using standard acrylamide conditions (39 wt%), which revealed that any change in saccharide mobility must be down to the presence of the boronic acid and not to changes in gel morphology owing to the incorporation of an aromatic group.

The *ortho*-boronic acid was the least effective in terms of electrophoresis modulation and, owing to the higher synthetic yield of the *meta*-derivative, this compound was used in the majority of our early investigations.

Electrophoresis experiments with gel phases that did and did not contain boron (BASE and FACE techniques, respectively) were conducted and the separation of fluorophore[76],[77]-labeled saccharides was assessed. Figure 11 demonstrates a case where FACE performs poorly in separating a series of 2-AMAC-labeled saccharides, whereas BASE elicits notable differences between the mobilities of the saccharides probed. Because BASE allowed us to separate previously inseparable saccharides, BASE offered us the opportunity to probe the separation of more-complex analytes (discussed in the next section).

Pro-BASE (mP-AGE)

A protein that had been shown by van den Elsen and co-workers to inhibit the innate immune system is under development as a therapy for complement-mediated acute inflammatory diseases that contain a 25-residue N-terminal tag (MSYHHHHHHDYDIPTTENLYFQGAM);[78] mass spectrometry analysis of similar constructs that contain the same tag have been shown to be especially prone to 6-phosphogluconoylation (6PGL). Because such detection of a D-gluconolactone modification of N-terminal adducts is a particularly important analytical target, mass spectrometry shows an increase in mass of 258 Da, which represents 6PGL, and/or an increase in mass of 178 Da, which corresponds to the D-gluconolactone adduct that arises from dephosphorylation.[79] To test whether we could both detect and separate gluconylated protein, we reacted purified protein with gluconolactone and followed the reaction by electrophoretic analysis at various time intervals by using standard polyacrylamide gel electrophoresis (PAGE) and protein BASE (Pro-BASE) with this specific system published as mP-AGE (methacrylamido phenylboronate acrylamide gel electrophoresis, Figure 12). After less than one minute, Pro-BASE showed a new band that was almost indistinguishable by the standard PAGE technique. The band intensified over 16 hours, as shown in Figure 12. What appears as a small shadow to the main 16.5 kDa band in a standard PAGE gel has an increased apparent molecular weight in the Pro-BASE system as a function of the boron content of the gel (tested over a boronic-acid-monomer-incorporation range of 0 to 3 wt%).

In this case, apparent molecular weight describes the apparent increase in the observed molecular weight of the boron-binding species (versus the molecular weight standards, M) that occurs as a function of boron content in the gel and the relative virtual molecular weight is the apparent mass-increase factor normalized to the actual weight. For example, in the present case, the virtual molecular weight is about 60 kDa, which corresponds to an almost four-fold increase in the apparent molecular weight over the real molecular weight. Over a range of boron-monomer-inclusion percentages tested, the retention (or virtual molecular weight) parameters of the band that is believed to correspond to glyconoylated protein is proportional to the boron content in the gel, thereby confirming that the new band is directly affected by boron. Furthermore, the glycated and glycosylated proteins (non-enzymatic and enzymatic addition of saccharides to proteins) could also be distinguished by using this technique, owing to the differing construction of the sugar's link to the protein; hence, separation is possible.[80] Going forward, it is envisaged that changes in the structures of glycoconjugates that may not necessarily be obvious from a mass/charge perspective could be probed by Pro-BASE and offer new potential identification regimes for biomarkers of diseases.

Conclusions

In this brief summary of a few key findings, we hope that readers will gain an appreciation of the wealth of potential for scientific discovery that remains in boronic-acid-mediated sensing. New techniques that are still in their infancy are described rather than more-established technologies.

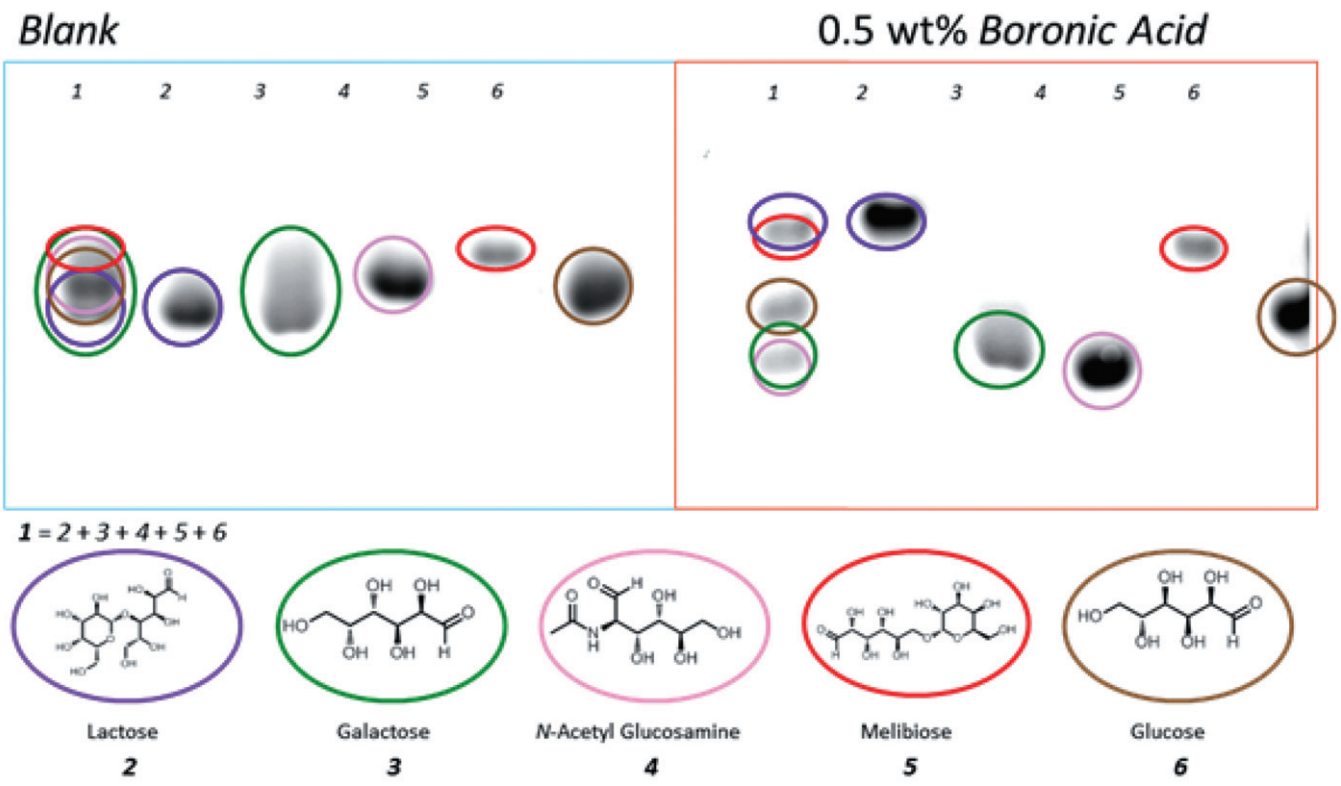


Fig. 11. Comparison of the electrophoretic separation of AMAC-labeled saccharides by using FACE and BASE (with and without boron, respectively).

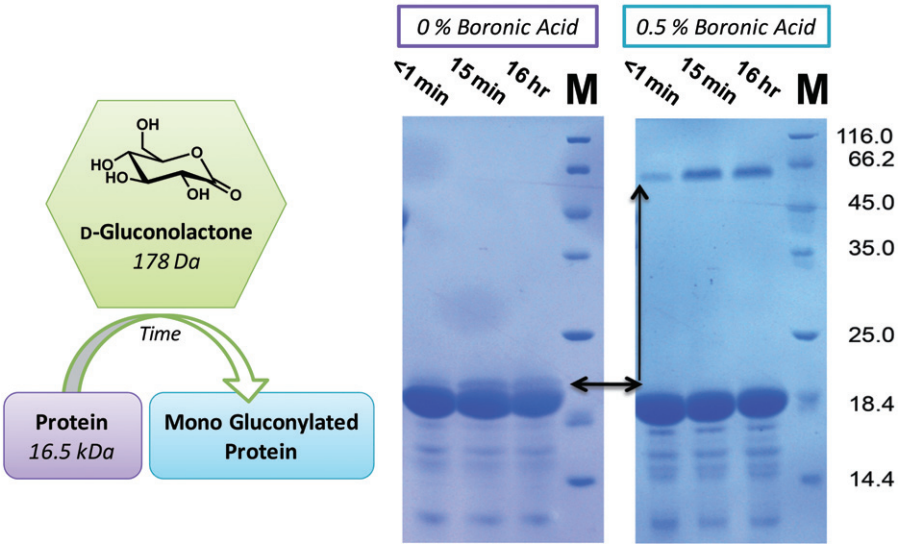


Fig. 12. Protein-boron-assisted saccharide electrophoresis (Pro-BASE) of a glyconoylated protein (right) versus the standard PAGE gel (left).

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