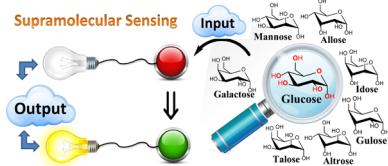


Glucose Sensing in Supramolecular Chemistry

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1. INTRODUCTION

The world is made up of fundamental elements, ions and molecules that continuously interact with each other through a series of chemical reactions. Thus, it is important to have a molecular-level understanding of these processes. Using this knowledge, scientists have developed research themes in the areas of chemical, material, physical, biological, and medical sciences. In order to better understand these processes, scientists have devoted much effort to developing effective tools for the understanding and exploration of these phenomena.

One of the most powerful tools available in the scientist's tool kit is molecular recognition and molecular assembly, which have

been extensively used to interact with and sense important species in natural systems, such as heavy metals, ions, saccharides, amino acids, peptides, explosives, and reactive oxygen/nitrogen species, to name but a few.¹ From a practical perspective, the concept of molecular recognition requires specific interactions between two or more molecules via noncovalent bonding interactions such as hydrogen bonding, metal coordination, hydrophobic forces, van der Waals forces, $\pi-\pi$ interactions, halogen bonding, and electrostatic and/or electromagnetic effects.² However, as the area has developed, molecular recognition and sensing systems have expanded to include covalent bonds and reaction-based systems.

Chemical sensors can be grouped as either biosensors or chemosensors. Biosensors make use of existing biological recognition elements. In many cases, selective biological receptors already exist for important analytes; therefore, modification of the receptor to include a signal transducer produces a biosensor.³ Chemosensors, on the other hand, use synthetically designed receptor units to produce selective sensors. The selectivity between the host and guest for chemosensors can be achieved via carefully geometrically matched electronic and polar elements. Our target as supramolecular chemists is to design and engineer synthetic receptors with selectivity for any chosen analyte, through the thoughtful choice of functional groups with appropriate three-dimensional placement. Such a concept is not new since the same process is clearly evident within the natural world, where biological systems have evolved to contain exquisitely assembled active binding sites, capable of selectively sequestering the desired target molecule.

An important aspect of synthetic receptors is that they can be, and often are, developed entirely from first principles. While this task appears daunting, many biomimetic synthetic receptors have been developed that are capable of mimicking the active sites in naturally occurring biological receptors. However, for this host–guest interaction to be of practical utility as a sensor, an additional element is required. The receptor system must establish a line of communication with the outside world. The ability to communicate the binding event with the outside world converts a receptor system into a sensor.

Therefore, for sensors to function, selective and specific binding interactions must occur between the host and guest, and the sensor must then simultaneously convert the binding events into a tangible output. These two aspects of a sensor allow it to communicate information on both the presence and location of important species in a measurable and quantifiable manner, effectively bridging the gap between events occurring on the molecular level back to the analyst on the macroscopic level.

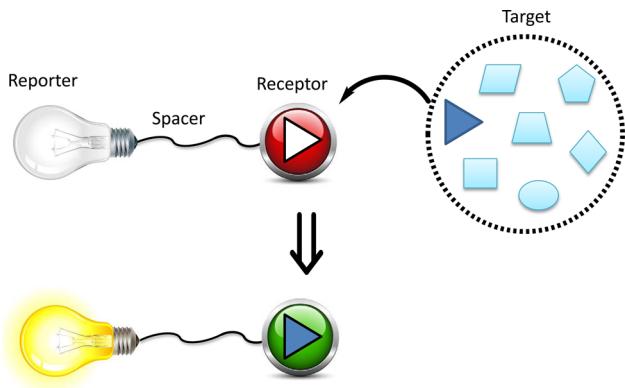
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For both biosensors and chemosensors, once target-specific recognition has been achieved, sensor development requires the incorporation of a signaling unit, which sensitively reports the presence of the bound guest. The choice of reporter depends on the monitoring environment, and some of the most widely used reporters are electrical and optical. The main requirement for these reporters is that a detectable signal must be produced in the presence of the guest (Scheme 1).

Scheme 1. Interaction between Guest Analyte and Host Receptor^a



^aRepresented as a triangular guest and host. Addition of a unit capable of generating a physical output signal in response to the binding event converts the receptor into a sensor. In this scheme an optical off-on response for an appended fluorophore is illustrated as an OFF light bulb switching to an ON light bulb, typical of the systems covered within this review. Image was prepared by use of light bulb (Kraska/Shutterstock) and red and green button images (Roman Sotola/Shutterstock).

2. SACCHARIDES AND CARBOHYDRATES

Carbohydrates are one of the four major classes of organic compounds in living cells. They are the main source of energy for our bodies, building blocks for polysaccharides, and components of other molecules, such as DNA, RNA, glycolipids, glycoproteins, ATP, etc. Carbohydrates include monosaccharides (e.g., glucose) and disaccharides (e.g., sucrose), which are often called sugars, and large carbohydrate molecules (e.g., starch and cellulose), known as polysaccharides.⁴

In order to maintain consistency throughout this review, the general term saccharide is used to describe all polyhydroxylated carbohydrates.⁵ Saccharides, which are the products of photosynthesis, account for the most prolific class of organic compounds found on the surface of the Earth. In their most pervasive roles, they endow nature with structural rigidity, in the form of cellulose, and they also act as the energy store (starch and glycogen) that sustains life.⁶

2.1. Diabetes Mellitus

The disease of diabetes refers to a medical condition where a patient suffers from inadequate control of blood glucose concentrations, which can lead to serious complications like heart disease, stroke, damage to the kidneys and nerves, limb amputation, and blindness.⁷ These diabetic-related conditions occur due to uncontrolled blood glucose levels over a prolonged period of time, and it is therefore essential that patients are diagnosed, treated, and monitored to maintain blood glucose levels within healthy limits. Treatment of diabetes involves a

combination of diet control, exercise, home blood glucose testing, medication, and insulin injection;⁸ however, current regimes are far from perfect and rely heavily on informed patient compliance. It is also important to carry out glucose monitoring of patients who are prediabetic or have gestational diabetes, since changes to diet, lifestyle, and medical intervention can reverse disease progression. The aim of all diabetic treatments is to maintain a patient's blood glucose levels within healthy physiological boundaries, and this is normally achieved by twice-daily invasive blood analysis (e.g., painful finger-prick blood collection) using testing strips and glucose monitoring systems that require external calibration at least once a day.⁹ The development of calibration-free devices for glucose measurement is potentially groundbreaking, since it would remove human error associated with current calibration processes, remove the need for painful finger-prick blood tests, and bring the possibility of developing a closed-loop artificial pancreas for automated regulation of glucose blood levels one step closer.

Protein glycation has also been widely implicated with the development and progression of diabetes, which is caused by irreversible reaction of the amine groups of amino acids and proteins with excess glucose present in blood to afford advanced glycation end (AGE) adducts that are deleterious to health. The concentration of glycated hemoglobin (HbA1c) in blood is currently used as an indirect measure of average plasma glucose concentration over prolonged periods of time.¹⁰ Glycation mainly affects long-lived proteins, and since the life span of an erythrocyte is approximately 120 days, HbA1c concentrations reflect plasma glucose levels over a period of a few months.¹¹ In cases where glycemic control worsens over a short time period, levels of HbA1c are not considered to be an appropriate index of glycemic control. Furthermore, HbA1c measurements are affected by pregnancy and variant hemoglobin diseases that can shorten the life span of erythrocytes (e.g., anemia, chronic liver disease, diabetic nephropathy, etc.). Human serum albumin (HSA) contributes the largest proportion of protein in the blood and has a much faster turnover than hemoglobin. So it should be considered as an ideal complement to HbA1c as an indicator of the short-term status of glycemic control, since it is not influenced by disorders of hemoglobin metabolism. A device capable of measuring the ratio between HbA1c and glycated serum albumin would therefore provide a highly valuable ratiometric index of the effectiveness of both short-term and long-term glucose management.

It is becoming increasingly obvious that glycated proteins may be important biomarkers for other sugar-related noncommunicable disease states, such as atherosclerosis, autoimmune diseases, cancer, and Alzheimer's disease (AD). For example, some researchers have coined AD as type 3 diabetes, with many of the unexplained features of AD, such as cell death and plaques in the brain, being directly linked to abnormalities in insulin signaling.¹² Although it remains controversial whether the formation of AGEs in AD and other age-related diseases is a primary or secondary event, detection of specific proteins could provide a very useful method for early-stage diagnosis and monitoring of disease. Although these protein modifications accumulate in the normal process of aging, for the case of unchecked hyperglycemia, glycation compromises proteins throughout the body, with elevated AGE levels having been identified as key biomarkers of diabetes-related complications, such as retinopathy, nephropathy, neuropathy, and coronary artery disease.

Diabetes is a serious condition that can affect many parts of the body and is linked with many serious illnesses, such as heart disease and stroke, blindness, kidney failure, and lower-limb amputation. Also, it has been estimated that hypoglycemia and hyperglycemic crisis, high blood pressure, and high blood low-density lipoprotein (LDL) cholesterol can be triggered by diabetes.¹³ People suffering with diabetes could already have or develop many other complications or conditions, such as nerve disease, non-alcoholic fatty liver disease, periodontal (gum) disease, hearing loss, erectile dysfunction, depression, and complications during pregnancy.¹⁴ Research also indicates that diabetes mellitus (type 1 and type 2) is an established risk factor for periodontitis through a hyperinflammatory response to the periodontal microbiota and also impairs resolution of inflammation and repair.¹⁵

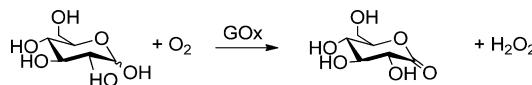
The 2014 National Diabetes Statistics Report indicates that diabetes affects 29.1 million people, 9.3% of the U.S. population, which includes 21.0 million people diagnosed and 8.1 million people undiagnosed.¹⁴ Recent data imply that approximately 20% of health care dollars go to treating people with diabetes.¹⁶

2.2. Home Blood Glucose Monitoring

Given the importance of sensing glucose to a significant percentage of the world's population, a large number of publications, including journals, Web sites, and books, have been produced. Given the cost, both personal and to society, a diverse range of medical devices has been developed and marketed.¹⁷ From a practical perspective, there are three approaches used for the sensing of glucose: these are based on (a) measuring the optical properties of fluorescent or labeled enzymes, their coenzymes, and cosubstrates; (b) monitoring the products of enzymatic oxidation of glucose by glucose oxidase; or (c) using synthetic boronic acid receptors as sensors.¹⁸ However, despite the huge economic and health drivers in this area, the development of methods for the continuous monitoring of glucose concentrations has only recently started to emerge.

Enzymatic glucose electrochemical sensors have attracted the most attention over the last 40 years due to their selectivity, simplicity, and sensitivity. Many clinical systems currently available for measuring blood glucose levels rely on the glucose oxidase enzyme (GOx, also commonly abbreviated to GOD or GO).

Scheme 2. Oxidation of D-Glucose to D-Gluconolactone, the Ring-Closed Form of D-Gluconic Acid, in the Presence of GOx



The majority of these blood glucose monitoring tools rely on the invasive withdrawal of blood, typically from a pricked finger, followed by application of the sample to an amperometric enzymatic test strip, allowing GOx to catalyze the oxidation of glucose to gluconic acid (Scheme 2).¹⁹

Early glucose monitors measured the production of hydrogen peroxide by oxidation at a single working electrode, as in eq 1. At a constant voltage, the current generated across the cell is proportional to the concentration of hydrogen peroxide, which is in turn proportional to the glucose concentration in the sample under investigation.



As this method of monitoring glucose is heavily dependent on the oxygen concentration, a dimethylferrocene mediator was developed. At a set potential of +160 mV, glucose is oxidized with concurrent reduction of the dimethylferricinium cation; the applied potential serves to oxidize and thus recycle the resulting dimethylferrocene back to the dimethylferricinium cation (Scheme 3).²⁰

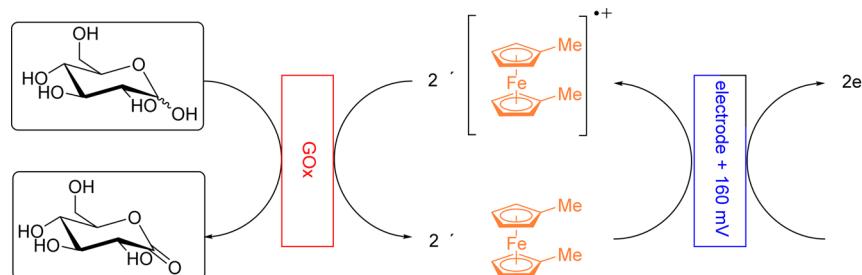
There can be no question that the availability of affordable enzyme-based home blood glucose monitoring has revolutionized the quality of life experienced by diabetics. However, there are some inherent limitations with an enzymatic approach. The systems have to be stored appropriately, they are specific only for a few saccharides, and in most cases they become unstable under harsh conditions and hence cannot be sterilized. In addition, other serious problems include low reproducibility, which is caused by sources of extraneous oxygen.

When the serious flaws described above are considered, nonenzymatic glucose sensors will probably become the next-generation glucose sensors for analytical applications. The Holy Grail of glucose sensing is the design of a continuous monitoring system, where the measurement of blood glucose could automatically trigger the release of insulin when an unhealthy concentration of glucose is exceeded.¹⁸ Toward that goal, much work has been focused on the development of synthetic sensors with the capacity to monitor saccharides under a broad range of environmental conditions and thus allow access to a wider spread of diagnostic applications.

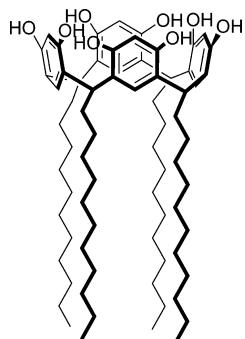
3. NON-BORONIC ACID GLUCOSE SENSORS

The biological importance of saccharides has resulted in a surge of interest in the development of synthetic molecular receptors with the ability to recognize saccharides. Our particular interest in developing saccharide receptors is primarily via the covalent interaction between boronic acids and diols. However, many other people have developed receptors using non-boronic acid approaches. For some excellent overviews of non-boronic acid-based systems the reader is directed to several reviews.²¹

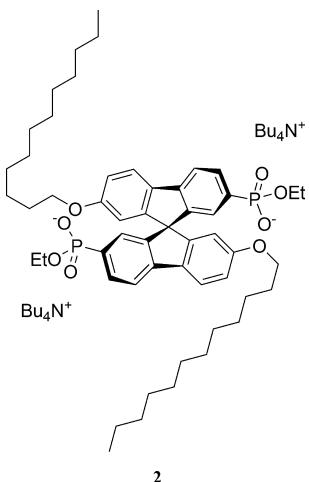
Scheme 3. Oxidation of Glucose with Concurrent Reduction of Dimethylferricinium Cation Mediator



The first synthetic saccharide receptor **1** was reported by Aoyama et al.²² in 1988. While the calixarene framework was too small to permit encapsulation of a saccharide, it was perfect for “face-to-face” recognition between the calixarene’s upper-rim hydroxyls and those of the saccharide. From a practical perspective, the system was not very good since, to form complexes of saccharides in water, high concentrations of saccharides were required (100 mM–1 M).

**1**

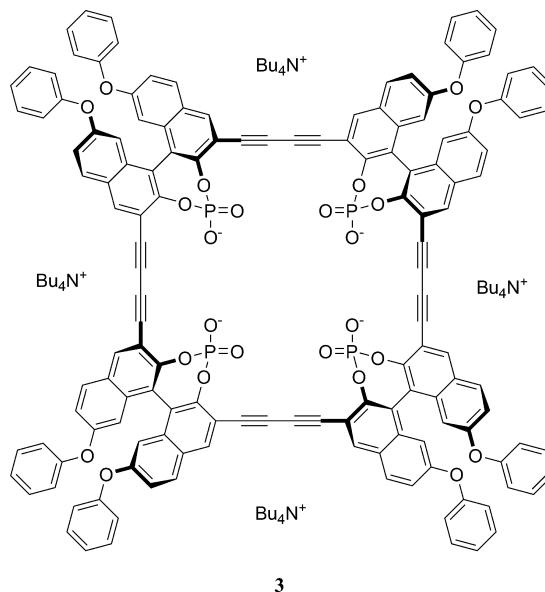
Das and Hamilton²³ have used phosphonate moieties as receptors for saccharides, such as the rigid chiral spirobifluorene **2**. The racemate was used directly, and although the exact nature of the hydrogen-bonding motif was not determined, excellent binding was observed with octyl glucosides in organic solvents. The binding constant (K_a) for sensor **2** with octyl β -D-glucopyranoside **5** was 47 000 M⁻¹ in deuterated acetonitrile.

**2**

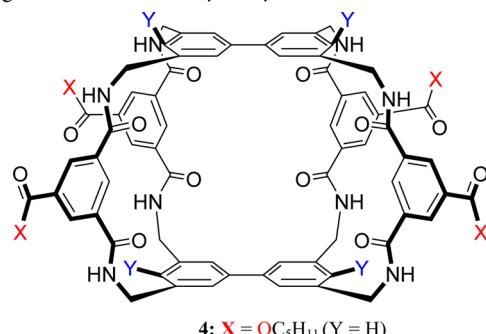
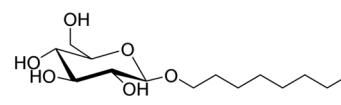
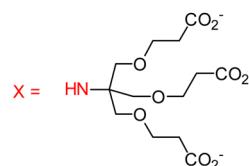
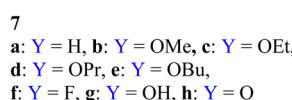
Diederich and co-workers²⁴ have developed a macrocycle appended with internal phosphate groups. Sensor **3** was shown to have good selectivity for saccharides such as octyl β -D-glucopyranoside **5**: the calculated interphosphate distance of 7.2 Å was designed to exclusively accommodate monosaccharides. A stability constant (K_a) of 5200 M⁻¹ was reported for the complexation of sensor **3** and octyl β -D-glucopyranoside **5** in 98:2 deuterated acetonitrile/deuterated methanol.^{24c}

The Achilles heel for the synthetic receptors described above is solvent competition and are therefore unable to measure glucose in aqueous media such as blood, urine, tear fluid and beverages. Because, monitoring saccharide interactions in water with these receptors is difficult, they are typically evaluated in aprotic solvents such as chloroform.^{21a,b}

Davis and Wareham²⁵ designed octaamide **4**. Their rigid and sophisticated architecture mimics nature in that the binding

**3**

pocket was designed to encapsulate the saccharide **5**. The planar hydrophobic surfaces of the dual diphenyl groups provide apolar contacts, and an array of amides provides favorable hydrogen-bonding interactions with hydroxyls of the saccharide.

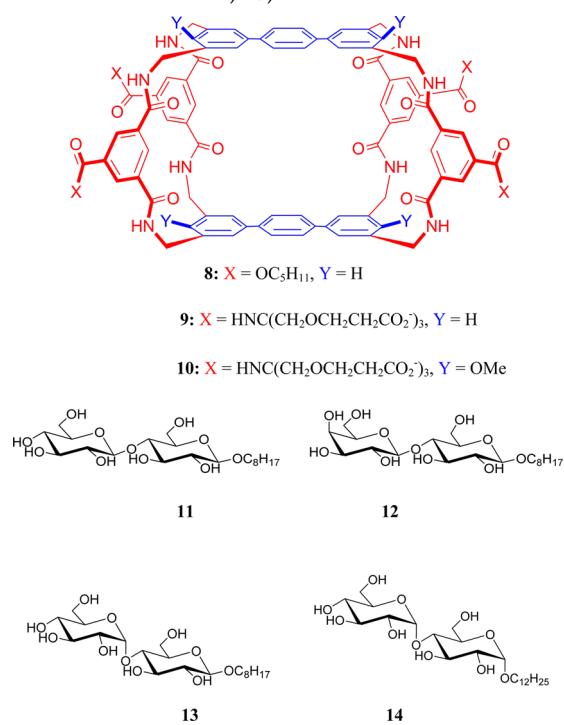
**4: X = OC5H11 (Y = H)****5****6 (Y = H)**

However, despite such elegant design, the reported stability constants (K_a) for **4** in deuterated chloroform are reduced significantly upon addition of competitive cosolvents. In the presence of octyl β -D-glucoside **5**, ~300-fold reduction was observed for the stability constant (K_a) of sensor **4** upon addition of 8% deuterated methanol. The stability constant (K_a) for sensor **4** with octyl β -D-glucopyranoside **5** was 300 000 M⁻¹ in

deuterated chloroform and $\sim 1000\text{ M}^{-1}$ in 92:8 deuterated chloroform/deuterated methanol. More recently, the dodeca-carboxylate version of this receptor, **6**, has been prepared and this system does bind D-glucose in water with a stability constant (K_a) of 9.5 M^{-1} .²⁶ This is much better than any of the previous systems, but the binding is still too weak in water for the system to be useful in detecting saccharides in biological systems.

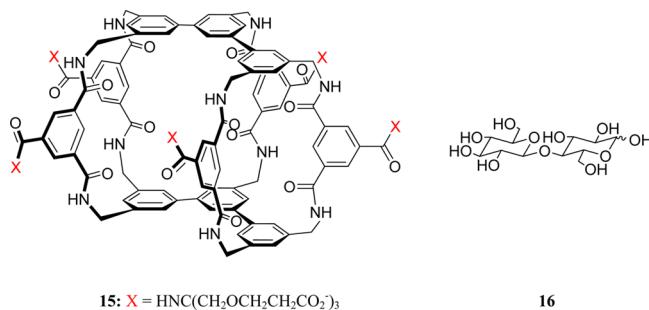
Subsequently, with modification of the substituents, Davis and co-workers²⁷ explored a series of new carbohydrate receptors **7a–h** without substantial modification of the core structure. The receptors were found to bind glucose with low affinity in water but were far more effective with β -GlcNAc, showing good selectivity and nearly millimolar affinity. Through detailed investigations, it was determined that the binding properties of the tricyclic monosaccharide receptors toward saccharides can be enhanced via modification of the biphenyl-based hydrophobic units. The role of CH– π interactions in carbohydrate recognition was also investigated, and changes in the biphenyl unit do alter the recognition through electronic effects. The results suggest that, with further modification and development, these systems could be used as part of practical blood glucose monitors.

Davis and co-workers²⁸ have also developed an extended tricyclic receptor **8**, which was designed to accommodate disaccharide guests. This is the first receptor capable of distinguishing between disaccharides through noncovalent interactions. In particular, the receptor displayed selectivity for disaccharide **11** as determined by an absence of NMR signals from the other disaccharides **12**, **13**, and **14**.

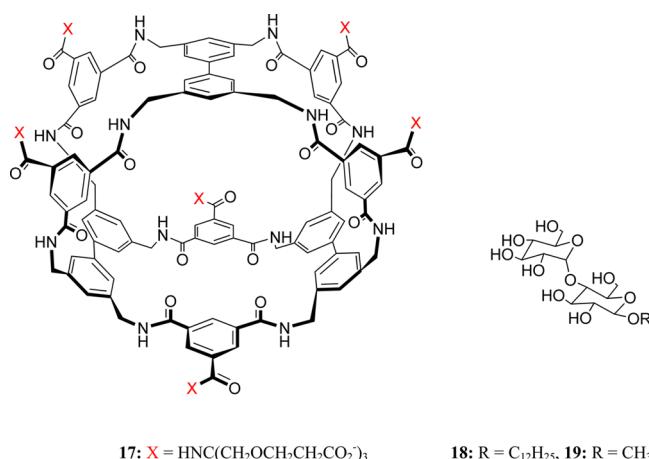


In order to overcome many of the weaknesses in the system, such as low binding constants and suboptimal selectivity, Davis and co-workers²⁹ have used a biomimetic recognition strategy to develop receptors for saccharides with all-equatorial stereochemistry (D-glucose, analogues, and homologues) and have now applied it to disaccharides such as cellobiose **16**. The new receptor **15** provides a realistic model for carbohydrate binding proteins. Using tetracyclic receptor **15** as a template, they developed another two examples, **9** and **10**, to improve the binding of all-equatorial disaccharides under biomimetic conditions.³⁰ The

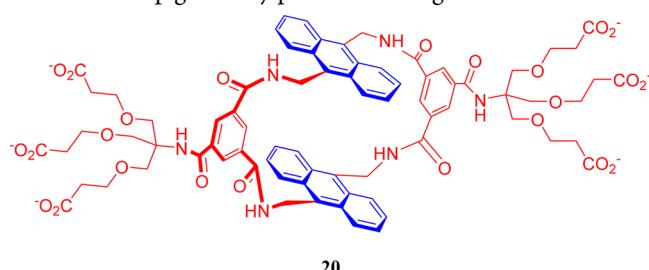
most important development was the proposal that induced fit or conformational selection was superior to defined and rigid preorganization in the design of saccharide receptors.



The macrotetracyclic receptor **17** was designed and developed for the selective detection of disaccharides and in particularly β -maltosyl.³¹ ¹H NMR and ITC (isothermal titration calorimetry) were used to measure the binding mode and K_a values, all of which demonstrated that **17** was an excellent saccharide receptor with selectivity toward β -glycosides **18** and **19**. These results indicate that selective tuning is possible in macropolycyclic polyamide hosts, clearly demonstrating that synthetic lectins with desired selectivity are potentially within the grasp of this class of receptors.

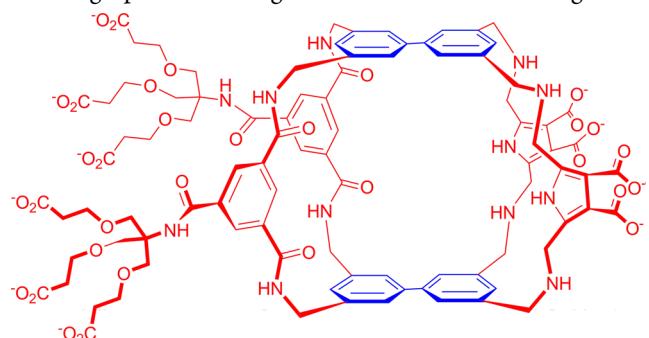


Recently, Davis and co-workers³² reported a simple and accessible receptor **20**, available in just five synthetic steps from commercially available starting materials, which is a dramatic improvement on the synthetic routes required for previous receptors. More importantly, the simplicity and accessibility of the receptor does not hamper performance as a glucose-selective receptor. The receptor displayed excellent signaling properties via changes of anthracene fluorescence upon saccharide binding. Therefore, receptor **20** with anthracene units as hydrophobic surfaces clearly demonstrated that this class of receptor could be used to develop genuinely practical blood glucose sensors.



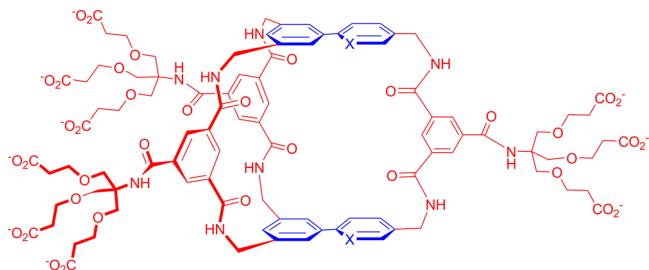
Joshi and Davis³³ also reported a related receptor **21**, which uses a new spacer, 2,5-bis(aminomethyl)pyrrole, with an

alternative (A-D-A) set of H-bonding valences. The modified spacer leads to substantial changes in binding selectivity, including a preference for glucose over other saccharide guests.



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A new bicyclic architecture, **22**, was developed by Davis and co-workers³⁴ with larger entrances to the receptor cavity than the earlier tri- or tetracyclic systems. The system retains the ability to bind saccharides in water, despite increased flexibility, and is particularly effective for sterically demanding saccharides. They also evaluated a receptor where the *p*-phenylene units were replaced with 2,5-linked pyridylene **23**. Sadly, the new structure resulted in a general lowering of affinities toward all saccharide guests.



22: X = CH

23; $X = N$

He and co-workers³⁵ developed self-assembled cerium metal-organic tetrahedrons for the luminescent sensing of saccharides (24, Scheme 4). The tetrahedral structure includes amide groups, which act as binding elements and signal transducers. The structure of the self-assembled system was confirmed by single-crystal X-ray structural analysis. However, saccharide recognition was possible only in mixed organic solvents and resulted in only low sensitivity and selectivity. The development of these systems into practical systems is therefore limited.

Yan and co-workers³⁶ have conjugated glucose oxidase (GOD) with phosphorescent Mn-doped ZnS quantum dots (QDs) by use

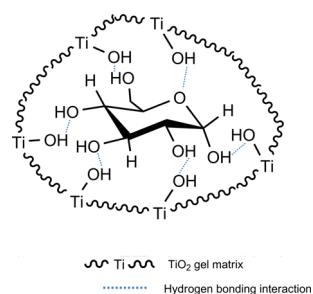


Figure 1. TiO₂ gel cavity imprinted for D-glucose.

of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC)/N-hydroxysuccinimide (NHS) as coupling reagents (Scheme 5). Glucose biosensing was possible with the system due to effective quenching of the room-temperature phosphorescence (RTP) of Mn-doped ZnS QDs by the H₂O₂ generated from GOD-catalyzed oxidation of glucose. Furthermore, the biosensor was applied for determination of glucose in serum samples without the need for any elaborate sample pretreatment.

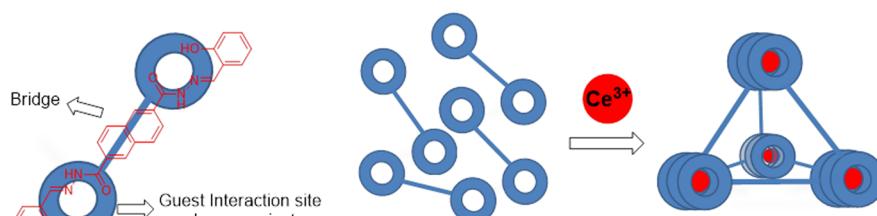
Qu and co-workers³⁷ determined that carboxyl-modified graphene oxide (GO-COOH) has peroxidase-like activity and can catalyze reaction of the peroxidase substrate 3,3',5,5'-tetramethylbenzidine (TMB) to produce a blue color in the presence of H₂O₂ (Scheme 6). This strategy is both simple and cheap, while also being highly sensitive and selective for the detection of glucose. The method has been applied to the detection of glucose in blood and fruit juice samples.

Another similar example was reported by Li and co-workers,³⁸ where positively charged gold nanoparticles, (+)-AuNPs, possess intrinsic peroxidase-like activity and can catalyze oxidation of peroxidase substrate 3,3,5,5-tetramethylbenzidine (TMB) by H₂O₂ to produce a blue color in aqueous solution, hence providing a simple approach for colorimetric detection of H₂O₂ and glucose (Scheme 7).

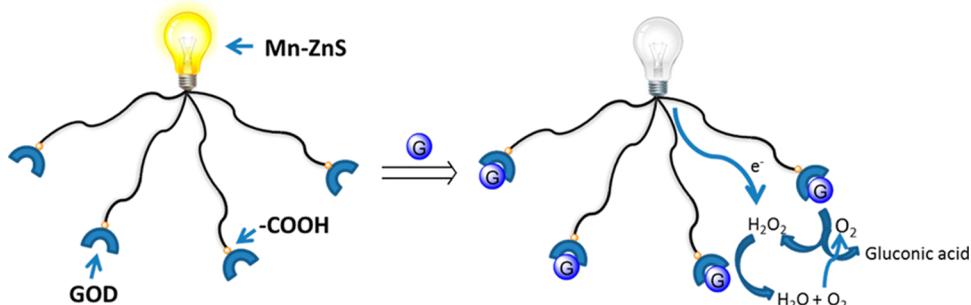
Composite nanofilms have been prepared from D-glucose with titanium *n*-butoxide, $Ti(O-nBu)_4$, on a quartz crystal microbalance (QCM) electrode via a surface sol–gel process (Figure 1).³⁹ Once the D-glucose template is removed, the binding behavior of the template with other monosaccharides (D-mannose, D-galactose, and D-fructose) was examined by QCM measurement. The D-glucose-imprinted TiO_2 gel film can sensitively detect monosaccharides via a mass increase. The largest binding was with D-glucose, with the largest binding ratio of 2.3 ($\lambda = M_{imp}/M_{nonimp}$, mol/mol) between the imprinted and nonimprinted films. The binding efficiency with other monosaccharide guests was 44–68% less than that determined for D-glucose.

Janowski and Severin⁴⁰ have used a metal-based indicator displacement assay for the fluorometric sensing of saccharides in

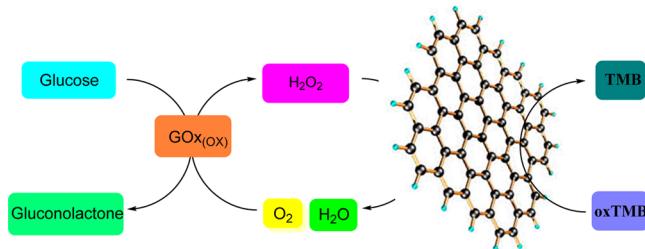
Scheme 4. Cerium-Based Self-Assembled Tetrahedrons



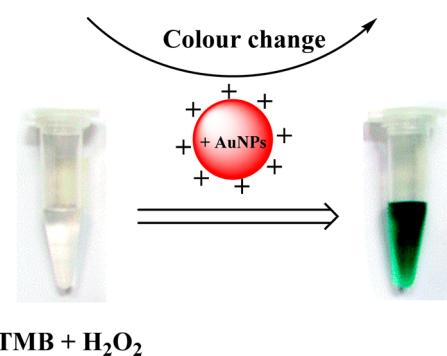
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Scheme 5. GOD–Mn-Doped ZnS QD Bioconjugate for Glucose Sensing^a

^aPrepared by use of light bulb images (Kraska/Shutterstock).

Scheme 6. Glucose Oxidase (GOx) and GO-COOH-Catalyzed Reactions for Colorimetric Detection of Glucose^a

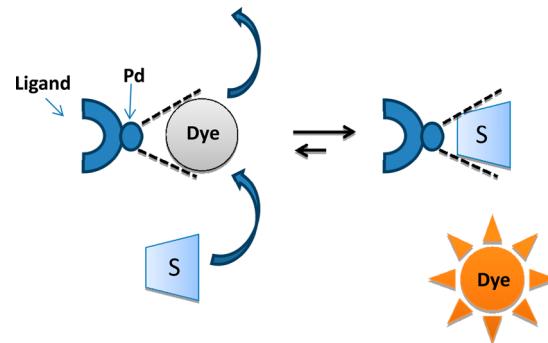
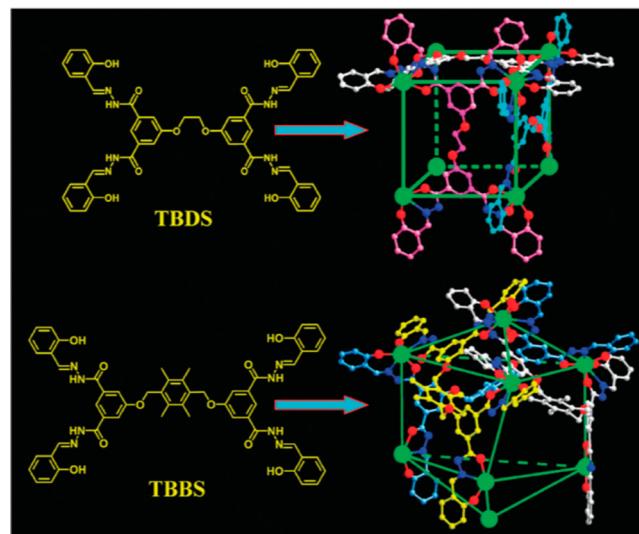
^aPrepared by use of graphene images (Andrew R. Barron and Zheng Yan), Characterization of Graphene by Raman Spectroscopy, OpenStax CNX, June 29, 2010; <http://cnx.org/contents/f06226c5-c2a4-4798-9c75-b016acea73cd@2>.

Scheme 7. Oxidation of TMB by H₂O₂ in the Presence of (+)-AuNPs: A Simple Approach to Colorimetric Detection of H₂O₂ and Glucose^a

^aReproduced with permission from ref 38. Copyright 2010 Royal Society of Chemistry.

water at neutral pH (Scheme 8). A fluorescent dye is bound to a Pd(II) complex and addition of the saccharide guest leads to a displacement of the dye, resulting in a fluorescence increase. The fluorescence changes can then be correlated to the type and concentration of the saccharide. However, the system's applicability is limited due to low chemoselectivity.

Using NOO tridentate chelators in a rationally designed ligand system, He and co-workers⁴¹ have developed a well-defined molecular cube, Ce-TBDS, and a bincoronal trigonal prism, Ce-TBBS, via self-assembly (Scheme 9). The Ce-TBBS system was employed for luminescent detection of saccharides. The system was used for detection of monosaccharides and disaccharides, and 1:1 stoichiometric host–guest complexes were formed. However,

Scheme 8. Interaction of Saccharides with a Pd/Dye Complex Leads to Dye Displacement and Increased Fluorescence Emission**Scheme 9. Self-Assembly Structures of TBDS and TBBS^a**

^aReproduced with permission from ref 41. Copyright 2011 Royal Society of Chemistry.

there are still many challenges for this system, such as low sensitivity, selectivity, and a limited understanding of the mechanism of action.

Acarbose 27 is an antidiabetic drug used to treat type 2 diabetes mellitus.⁴² Fukuhara and Inoue⁴³ have found that the hybrid Cur–PT complexes formed *in situ* between Cur 25 and 2,5-poly[3-(1-pyridinium)hexylthiophene] 26 (PyPT) in aqueous solution can be used to discriminate acarbose from 24 mono-, di-

tri-, tetra-, and pentasaccharides (Figure 2). The long wavelength employed (>400 nm), high selectivity, and low detection limit (1 μM) make the system attractive for diagnosis and therapy in diabetes research.

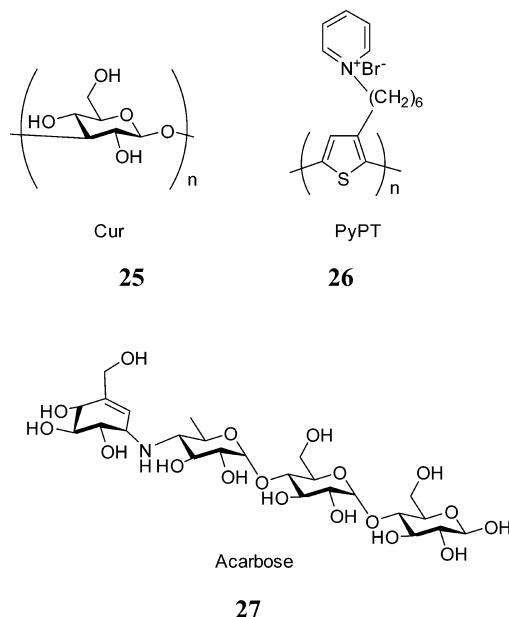


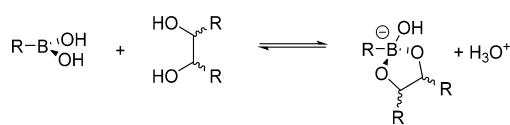
Figure 2. Structures of Cur, PyPT, and Acarbose.

4. BORONIC ACIDS FOR SENSING APPLICATIONS

Alkyl- and arylboronic acids both have been accessible for over 150 years. The most widely used method for their preparation is the reaction of organometallic reagents (Grignard or organolithium reagents) with trialkyl borates. The interaction of boronic acids with saccharides^{44,45} or anions^{45c,46} has been intensively studied, and boronic acids are employed for applications as varied as NMR shift reagents,⁴⁷ functional polymers,⁴⁸ and molecular self-assembled materials.⁴⁹ Sensor systems for reactive oxygen and nitrogen species (ROS and RNS) have also been developed that are based on oxidative removal of the boronic acid group.⁵⁰

Cyclic boric and boronic esters are formed when boronic acids react with saccharides (Scheme 10). Boronic acid-based receptor

Scheme 10. Cyclic Boronate Ester Formation Is Rapid and Reversible



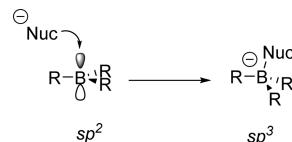
design originates with the seminal work of Lorand and Edwards,⁵¹ where the pH drop observed upon addition of saccharides was used to determine saccharide affinity. When 1,2-, 1,3-, or 1,4-diols react with boronic acids to form cyclic boronate esters (5-, 6-, or 7-membered rings) in aqueous medium, the acidity is enhanced.^{45c,48d} Water and phenylboronic acid form a tetrahedral boronate, causing the release of a proton and increase in acidity (K_a). Typically, phenylboronic acids have pK_a values between ~8.7 and 8.9, with $pK_a = 8.70$ in water at 25 °C.⁵²

Saccharide recognition via boronic acid complex (or boronic ester) formation frequently relies on the interaction between a

boronic acid (Lewis acidic) and a neighboring tertiary amine (Lewis basic). The nitrogen–boron (N–B) interaction and its nature have been much disputed (especially in an aqueous environment), but it is clear that an interaction of some sort exists, offering two main advantages:⁵³ (1) The interaction enhances binding at neutral pH (by enabling tetrahedral boronate formation), which is very important in the development of receptors with practical utility. (2) Saccharide binding strengthens the N–B interaction (caused by an increase in Lewis acidity of the boron upon saccharide binding), resulting in modulation of the fluorescence of nearby fluorophores (since the fluorescent photoinduced electron transfer (PET) from the nitrogen is controlled by the strength of the N–B interaction).

It is also worth highlighting that the Lewis acidic nature of boron has resulted in the development of receptors and sensors for anions (Scheme 11).^{45c,46}

Scheme 11. Change in Geometry at the Boron Center upon Nucleophile (Anion) Binding



The interaction of boronic acids with Lewis basic species in buffered solutions produces binary (boronate–Lewis base) complexes and also ternary (boronate–Lewis base–saccharide) complexes.⁵² These complexes persist into acidic solution, and under certain stoichiometric conditions they can become the dominant species in solution. Therefore, when fluorescence experiments are conducted in solutions buffered with Lewis basic components, a “medium dependence” is observed due to the formation of these Lewis base adducts. These complexes result in a reduction of the free boronate and boronic acid concentrations and reduce the observed stability constants (K_{obs}).

From a practical perspective, the buffer should be chosen so as not to overpower the system being investigated. Serendipitously, phosphate buffer (pH 7.5) does not significantly modify the binding constants of the complexes under investigation. Therefore, provided that one takes note of the conditions under which observed stability constants (K_{obs}) have been measured, spectrophotometrically obtained binding constants can provide useful information for the development of sensory systems using boronic acid-based receptors.

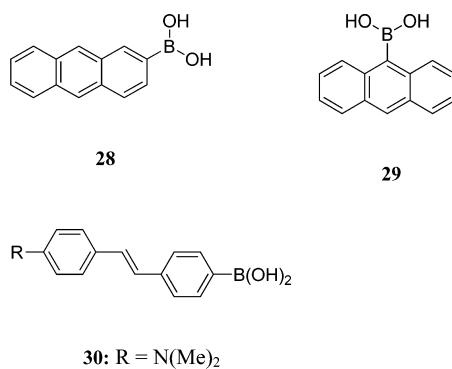
The fast and stable bond formed between boronic acids and diols, resulting in boronate esters, can be used to build dynamic and reversible molecular assemblies. For boronate esters, the covalent B–O bonds are very stable, but under certain conditions or under the action of certain external stimuli their formation is reversible.^{49d–f} This dynamic covalent nature of boronic acids and Lewis acid-base interactions with structure-directing potential has resulted in the use of boronic acids in a variety of self-organizing multicomponent systems.⁴⁹

4.1. Fluorescence for Sensing Applications

The use of fluorescence as a signal to report molecular recognition events was first reported during the early 1980s with the synthesis of calcium fluorescent indicators by Tsien and co-workers.⁵⁴ Fluorescence sensing is an excellent method for the detection of analytes with high sensitivity and selectivity, provided the sensor is designed appropriately. Fluorescent probes have been successfully developed for a large number of biomolecules

including glutamate, acetylcholine, glycine, aspartate, and dopamine.⁵⁵ Fluorescence is particularly useful to directly interrogate cell chemistry and to monitor function.⁵⁶ Fluorescence is also important for environmental analysis and the early detection of toxic metals, such as mercury, lead, and cadmium.⁵⁷ Additionally, applications include reliable, real-time biological and chemical sensing of explosives and hazardous chemicals in land mines⁵⁸ and chemical weapons.⁵⁹ These many positive features make fluorescence one of the most powerful transduction mechanisms to report on chemical recognition events.

In 1992, the first fluorescence-based saccharide sensor using boronic acids was developed by Yoon and Czarnik.⁶⁰ The excited-state internal charge transfer (ICT) sensor **28** consists of a boronic acid unit directly linked to the fluorescent unit (anthracene). With ICT systems, heteroatoms or strongly inductive functional groups can cause an imbalance in the electron density across the excited state of the molecule. This reversible charge separation creates an enhanced dipole moment, increasing the favorable dipole–dipole interaction of the fluorophore with the surrounding solvent shell. This environmentally dependent stabilization can lead to dramatic changes in the wavelength of the emission band and as such has found great application in the field of sensing. In the case of Czarnik's seminal system, the addition of saccharides to **28** resulted in a 30% decrease in the intensity of the fluorescence emission (on–off fluorescence sensor). The fluorescence modulation is believed to be caused by changes in the electronic properties accompanying rehybridization at the boron from sp^2 to sp^3 . Yoon and Czarnik⁶¹ also investigated 9-anthrylboronic acid **29**; however, this isomer resulted in smaller changes in fluorescence emission, attributed to unfavorable peri interactions that are predicted for the 9-position due to the adjacent hydrogens.



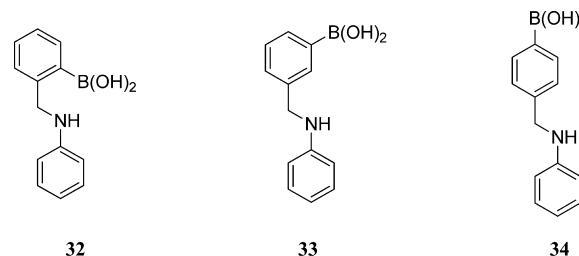
30: R = N(Me)₂

31: R = CN

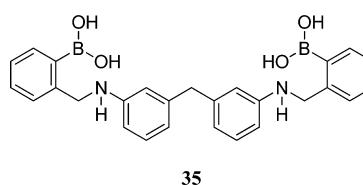
Subsequently, an excellent example of the design of a boronic acid-based ICT saccharide receptor was reported by Lakowicz and co-workers,⁶² who have developed a range of stilbeneboronic acid derivatives. The neutral (sp^2 -hybridized) boronic acid acts as an acceptor group, while the anionic (sp^3 -hybridized) boronic acid behaves as a donor group. For 4'-(dimethylamino)stilbene-4-boronic acid **30**, the dimethylamino moiety is the donor group. When sp^2 -hybridized, the boron is the acceptor, and excited state ICT occurs between the amino donor group and boron, causing a red shift of the emission wavelength of the sp^2 species. Upon hybridization of the boron to sp^3 , the acceptor properties are removed, resulting in a loss of ICT for the sp^3 excited-state species, and the emission wavelength of the fluorophore shifts to a higher energy. Since the sp^3 -hybridized species is unable to lower the excited-state energy by the same mechanism available with the sp^2 -hybridized species, this causes a significant change in the

observed emission band. The upshot is that the sp^2 to sp^3 conversion increases the emission intensity and blue-shifts the emission wavelength by 45 nm. However, with 4'-cyanostilbene-4-boronic acid **31**, the cyano group is an acceptor unit. In this case sp^2 -hybridized boron is an acceptor and no excited-state ICT is possible. In this situation, sp^3 boron (a donor) facilitates ICT between the boron donor and cyano acceptor, and the emission wavelength is red-shifted. Interestingly, reversing the roles of the donor and acceptor units, as with sensor **31**, results in changes of intensity and emission wavelength that are similar to those observed with sensor **30** but at the opposite end of the spectrum. Hybridization changes from sp^2 to sp^3 result in a reduction of the emission intensity and red shift of the emission wavelength (40 nm).

For aniline-based sensor **32**, upon addition of fructose in aqueous solution, the emission peak was blue-shifted by 42 nm. The observed dual fluorescence was due to twisted internal charge transfer (TICT) in the case of neutral boronic acid and locally excited (LE) states in the case of anionic boronate.⁶³



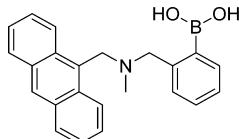
TICT arises in the neutral boronic acid **32** due to the structure containing a nitrogen–boron (N–B) interaction. This interaction has been confirmed by X-ray crystallography: the nitrogen lone pair coordinates to the boron with the N–B bond perpendicular to the anilino π -system (in protic systems the direct N–B bond is replaced by the solvent-inserted system). This stabilization of the excited state is lost as soon as the boronic acid is converted to the anionic boronate, either through pairwise complexation of a saccharide guest or through an increase in the pH of the solution. However, for **33** and **34**, the increased N–B distance of the meta- and para-substituted boronic acids prevents N–B interaction and stabilization by TICT. This concept was extended to produce sensor **35**, designed to exhibit glucose selectivity via the introduction of a second, correctly positioned boronic acid recognition site.⁶⁴



35

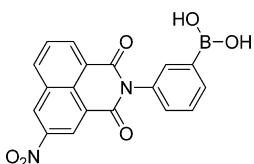
A very important aspect of boronic acid-based ICT sensors, when considering their potential for development as real-world sensors, is to remember that they are strongly affected by changes in pH. The next class of fluorescence systems for saccharides to be discussed is photoinduced electron transfer (PET) sensors, which are probably the most important class of boronic acid-based fluorescence sensors. Importantly, PET sensors such as **36** are not significantly affected by changes in pH, since with PET sensors the interaction of tertiary amines (Lewis bases) with the boron of *o*-methylphenylboronic acids (Lewis acids) removes the pH sensitivity of the boronic acid as well as producing fluorescence off-on or turn-on sensors for saccharides. While the exact nature

and structure of the amino group and boronic acid (N–B) interaction is disputed,^{45i,53,65} the existence of the interaction does result in reliable binding over a wide pH range (i.e., lower pK_a of the boronic acid).⁶⁶



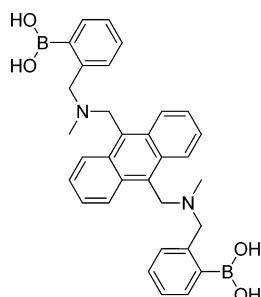
36

The naphthalimide fluorophore is particularly useful for the development of sensors since it has long excitation and emission wavelengths. Heagy and co-workers,⁶⁷ in seminal work, developed sensors using naphthalimide fluorophore 37, while a naphthalimide-based system that could produce long-wavelength fluorescence changes upon addition of saccharides was also developed by Mohr and co-workers.⁶⁸ Several water-soluble long-wavelength naphthalimide systems were designed by Wang and co-workers,⁶⁹ with the best system producing 2-fold fluorescence enhancement upon saccharide binding. The search for longer wavelength fluorescent boronic acid-based sensors led Mohr and co-workers⁷⁰ to develop a series of fluorescent hemicyanine systems; the best binding constant was obtained for D-fructose (280 M⁻¹).



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The monoboronic acid and fructose selective sensor 36 was enhanced by introduction of a second receptor to prepare a diboronic acid sensor 38.⁷¹ This system retained the advantage of utilizing PET to produce an off-on response to saccharides and introduced a potentially selective recognition site (two boronic acid receptors). The structural engineering proved very successful, and by “luck” the arrangement of the two boronic acid groups produced a selective binding pocket for glucose.



38

Saccharide sensors based on conventional PET (a-PET, with fluorophore as the electron acceptor), such as 38, have been extensively employed in the development of fluorescence sensors for saccharides. More recently, sensors based on reverse PET (d-PET, fluorophore as the electron donor) have been developed by Zhao and James and co-workers;⁷² here, excited-state electron

transfer to the protonated amine/phenylboronic acid unit from the fluorophore occurs. Unlike normal PET (a-PET) systems, these sensors work better at lower pH. It has long been realized that the selectivity of boronic acid sensors for saccharides can be improved by considering the exact structure of the guest. However, even with a long research history into the structural nature of boronic acid-based saccharide complexes, the rapid isomerization of monosaccharides in water precludes development of a simple structural model for saccharide complexes. The hemiacetal ring of monosaccharides is easily cleaved in water and can re-form, producing rings of different sizes and anomeric configurations. The complex equilibrium of linear, furanose, and pyranose forms coupled with α - and β - anomers dramatically increases the total number of plausible structures.

Analysis of the D-glucose complex of diboronic acid 38 represents the first detailed investigation of a system containing two boronic acid receptors. ¹H NMR analysis of the D-glucose complex in deuterated methanol pointed to a complex with the α -pyranose form binding to the 1,2 and 4,6 positions of D-glucose (Figure 3).⁷¹

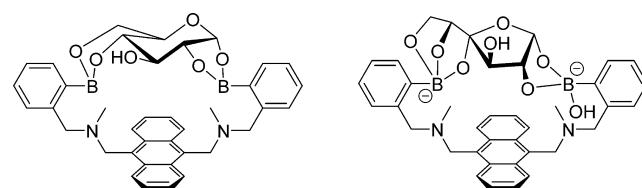
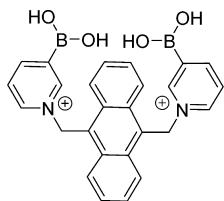


Figure 3. (Left) Initial 1,2:4,6 complex formed for sensor 38 and D-glucose in MeOD. (Right) 1,2:3,5,6 complex observed between sensor 38 and D-glucose in basic aqueous medium.

However, this proved to be only part of the story; the ¹³C-1 and ¹³C-6 labeled D-glucose experiments of Eggert and Norrild and co-workers,⁷³ who monitored the ¹J_{C1-C2} and ¹J_{C5-C6} coupling constants, were required to fully explain the structure of the D-glucose complex. Their results indicated that the previous ¹H NMR assignment was correct; however, the interpretation was valid only for initial complex formation and under anhydrous conditions. When the system was monitored over time, they concluded that α -D-glucopyranose isomerized to the α -D-glucofuranose form. This process was slow in deuterated methanol; complete disappearance of the original α -D-glucopyranose signals was observed after 8 days. However, if water was added to the system, isomerization occurred rapidly, and after 10 min in a 1:2 water/methanol solution, isomerization was complete. Therefore, for sensor 38 it was decided that in water the complex rearranges to the thermodynamically more stable 1,2:3,5,6 bound α -D-glucofuranose complex (Figure 3).

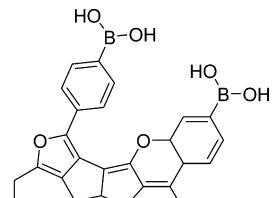
A novel boronic acid turn-on fluorescence system 39 has been prepared by Norrild and co-workers:⁷³ upon saccharide binding, quenching by the pyridine groups is reduced, resulting in an enhanced fluorescence output. The structure of the saccharide complex was found to contain α -D-glucofuranose bound with the boronic acid groups at the 1,2 and 3,5 positions. The furanose structure was determined from ¹H and ¹³C NMR data and information from ¹J_{C-C} coupling constants.

A computer-aided approach was used by Drueckhamer and co-workers⁷⁴ to construct sensor 40, which was specifically designed to complex the 1,2 and 4,6 positions of α -D-glucopyranose. The computational approach resulted in a molecular scaffold where two boronic acid groups were precisely positioned. The rigid and locked spatial architecture of sensor 40



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resulted in enhanced affinity and selectivity for D-glucose (400 times) compared to other saccharides. More importantly, the complex contained the pyranose form of D-glucose as determined by ^1H NMR analysis. Sensor **40** demonstrates that where two-point binding is achieved for boronic acids of fixed distances with enforced geometries, then specific isomeric forms of a particular saccharide guest can be selectively retained within the binding pocket.

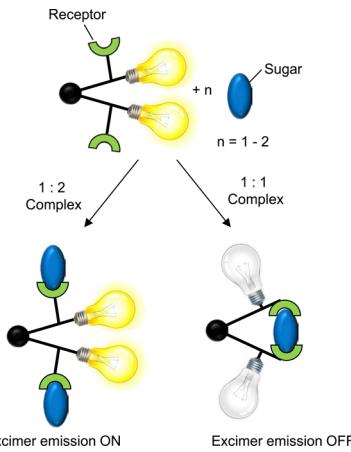


40

When one is looking to enhance and improve the selectivity of boronic acid-based receptors, pre-empting the structure of the guest species or the thermodynamic complex that forms is nontrivial, particularly because it is known that only saccharides with the ability to equilibrate the furanose and pyranose forms and a pair of hydroxyl groups (including the anomeric position) form strong complexes. Also, in aqueous solutions the furanose form of glucose is thermodynamically preferred. These observations make the research by Drueckhammer and others particularly poignant, since it indicates that in systems with two linked boronic acid receptor units the structure of the bound saccharide complex can be directed by the structure and geometry of the receptor.

Molecular tweezer **41** was conceived to selectively open for certain saccharides.⁷⁵ The fluorescence intensity at 377 nm of molecular tweezer **41** increases with increasing concentration of D-glucose, D-fructose, D-galactose, and D-mannose, while, the fluorescence intensity at 470 nm is different among the four saccharides. With increasing D-glucose and D-mannose concentration the 470 nm band decreases, and the intensity of the 470 nm band is invariant with added D-fructose. However, for D-galactose an initial quenching of the 470 nm band at low concentrations is followed by fluorescence increase at higher concentrations. In the case of the saccharides D-glucose, D-galactose, and D-mannose, a cyclic 1:1 structure is formed (Scheme 12). Quenching of the intramolecular excimer emission at 470 nm is then explained due to separation of the pyrene fluorophores upon saccharide binding. However, with galactose the observed behavior is more complex and indicates the formation of a 1:1 complex at low concentration and a 1:2 complex at higher saccharide concentrations. With D-fructose, only the noncyclic 1:2 complex forms even at low saccharide concentrations (Scheme 12).

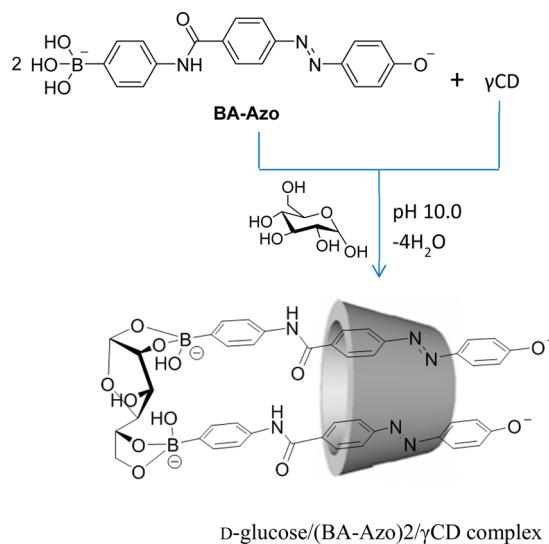
One of the most pursued molecular sensors based on boronic acid is a glucose-selective system. Toward that goal, many reports dealing with synthesis and characterization of novel fluorescent recognition systems with new mechanisms are constantly being

Scheme 12. Binding Modes for Molecular Tweezer **41**^a

^aPrepared by use of light bulb images (Kraska/Shutterstock).

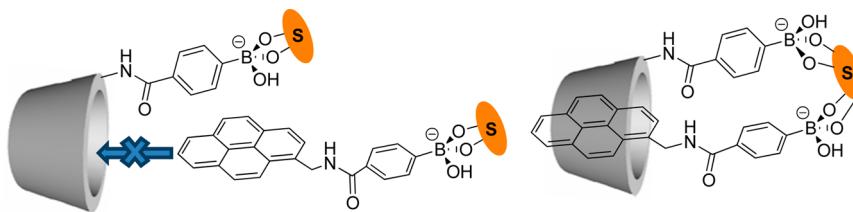
published. For example, pyrene-based boronic acids were shown to bind glucose⁷⁶ and monosaccharides,⁷⁷ and a boron-dipyrromethene (BODIPY) fluorophore was shown to bind glucose at physiologically important concentrations.⁷⁸ Also, a series of isoquinolinylboronic acids have been shown to have particularly high affinity for diol-containing compounds at physiological pH. In particular, 8-isoquinolinylboronic acid displayed good binding affinity toward D-glucose ($K_{\text{obs}} = 46 \text{ M}^{-1}$).⁷⁹

Hayashita and co-workers⁸⁰ have taken a very interesting supramolecular approach to the development of glucose-selective diboronic acids. Monoboronic acid BA-Azo (Scheme 13) forms a

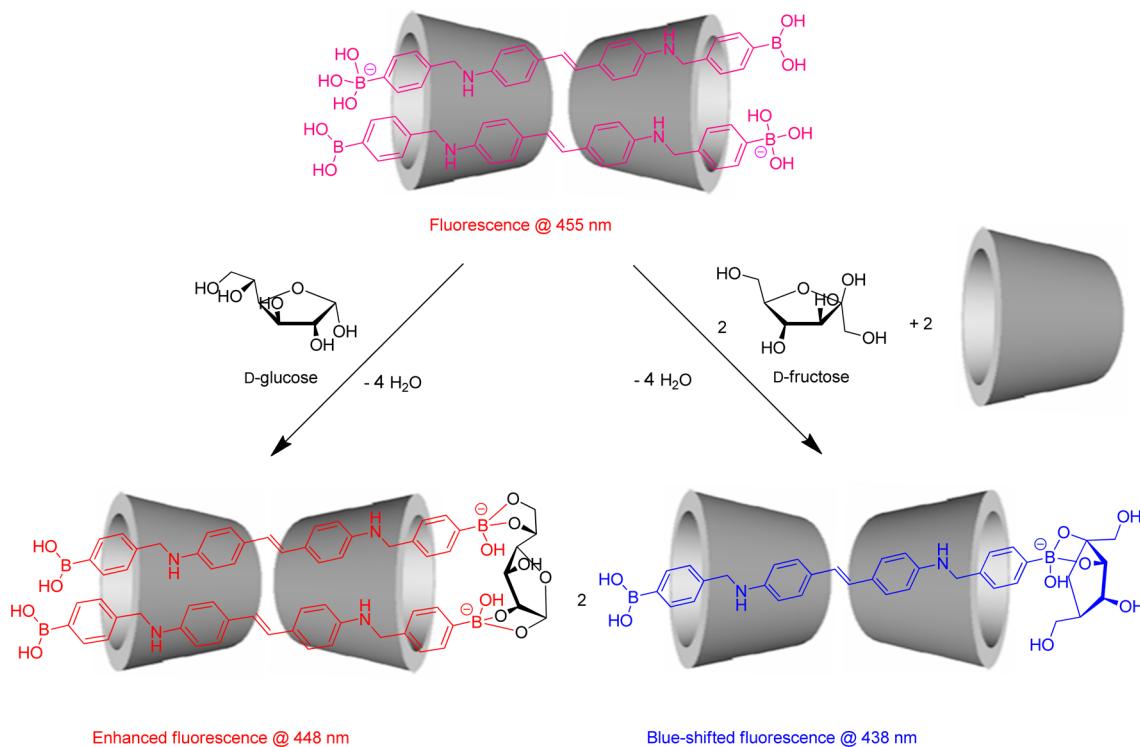
Scheme 13. 2:1 Inclusion Complex Formed for BA-Azo with γ -CD in the Presence of D-Glucose^aD-glucose/(BA-Azo)2/ γ CD complex

^aPrepared by use of cyclodextrin image (Hayashita/Sophia University).

2:1 inclusion complex with γ -cyclodextrin (γ -CD) in the presence of glucose. Formation of the inclusion complex can be followed by changes in the UV-vis spectra. Hayashita and co-workers⁸¹ have also elegantly designed another γ -CD system that exhibits selective fluorescence enhancements with glucose (Scheme 14).

Scheme 14. Hayashita γ -CD System: (left) Fructose and (right) Glucose^a

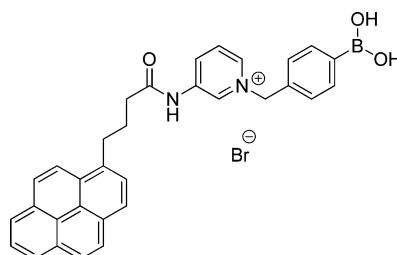
^aPrepared by use of cyclodextrin image (Hayashita/Sophia University).

Scheme 15. 2:2 STDBA- γ -CD Complex Formed with Glucose and Fructose^a

^aPrepared by use of cyclodextrin image (Hayashita/Sophia University).

Another interesting system using cyclodextrin has been developed by Jiang and co-workers,⁸² who used a hydrophobic stilbeneboronic acid (STDBA), two of which could be inserted into the cavity of γ -CD to form a fluorescent 2:2 STDBA- γ -CD ensemble (Scheme 15). The system displays a selective and sensitive response toward glucose in aqueous solutions and could be applied to the detection of glucose in artificial urine samples.

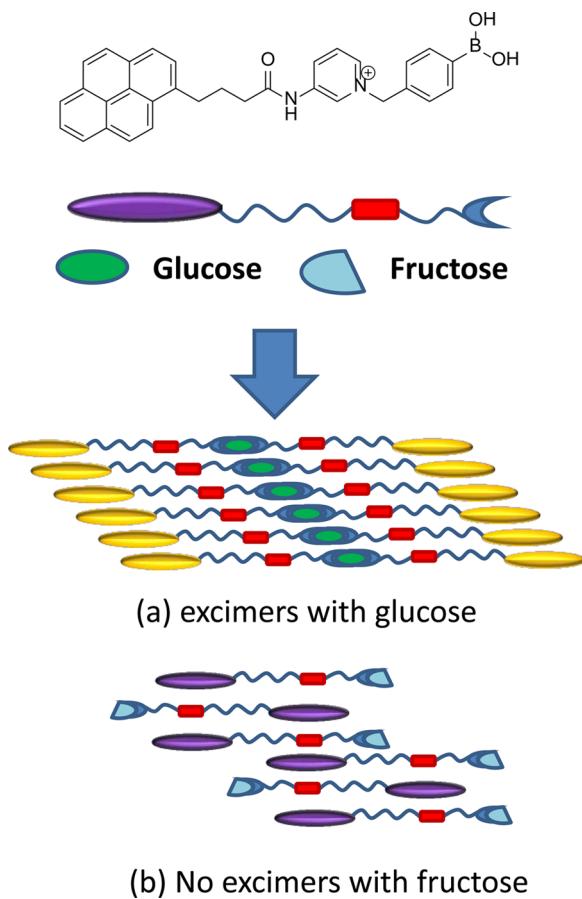
The problem associated with the higher affinity of simple monoboronic acids toward D-fructose over D-glucose was addressed in the elegant work by James and Jiang and co-workers⁸³ (Scheme 16, sensor 42). The specificity of boronic acid toward glucose was modulated by the inherent stoichiometry of glucose and fructose binding. By use of a pyreneboronic acid derivative, amorphous 1:1 conjugates were produced in the presence of D-fructose. However, highly ordered and fluorescent 2:1 conjugates were obtained in the presence of D-glucose. With this system, both boronic acid-diol interactions and π -stacking and aggregation of pyrene units were involved in the selectivity of the sensing mechanism. Additionally, D-fructose in a mixture of D-fructose and D-glucose could be knocked out by adding phenylboronic acid since it interacts strongly (selectively binds) with D-fructose.⁸³



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Tang and co-workers⁸⁴ have developed an exciting sensing system using tetraphenylethene (TPE) with two boronic acid units; the turn-on biosensor has a high specificity for D-glucose in aqueous solution (Scheme 17). The oligomerization reaction of two *cis*-diol units of glucose with two boronic acid units results in structurally rigid oligomers, resulting in a significant increase in the fluorescence intensity via aggregation-induced emission (AIE). This novel sensing process ignores saccharides with only one *cis*-diol unit and results in high specificity for glucose over its close isomeric cousins fructose, galactose, and mannose.

Scheme 16. (a) Highly Structured Aggregates Producing Excimer Fluorescence, Formed with D-Glucose, and (b) Amorphous Aggregates with No Excimer Fluorescence, Formed with D-Fructose



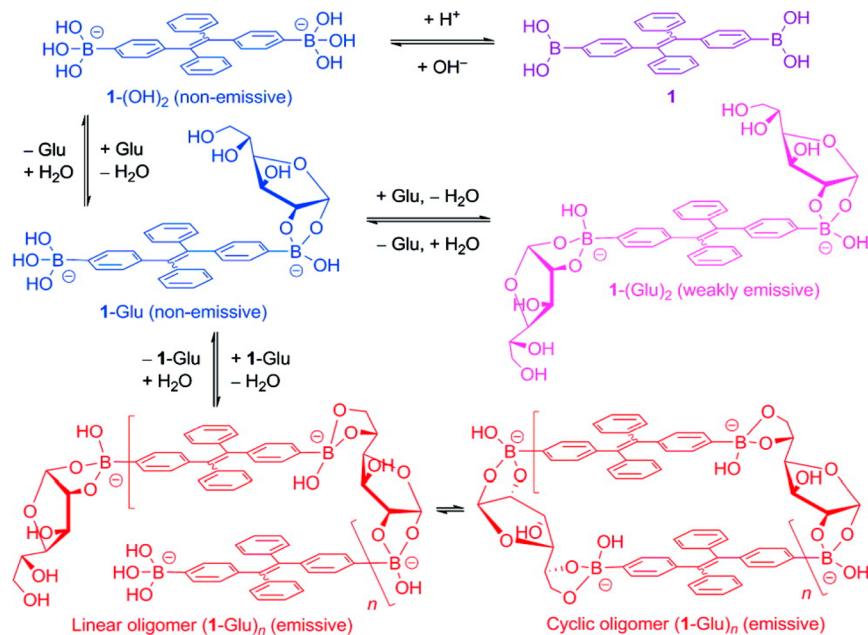
From the discussions so far, boronic acid-based sensor systems require more than one receptor to achieve saccharide selectivity.⁷¹ Simplified receptor design can be achieved by use of two boronic acid receptors in a modular system, where the linker and fluorophore units are varied independently. Such a modular approach to saccharide recognition has been championed by James and co-workers,^{75,85} Wang and co-workers,^{45a,86} Hall and co-workers,⁸⁷ and Singaram and co-workers.⁸⁸

Modular system 43, with two boronic acid groups and linker, allows the spacing between the boronic acids to be easily changed. The system also permits the fluorophore to be varied independently. For example, a series of PET sensors 44 have been prepared, containing two phenylboronic acid groups, a pyrene fluorophore, and a variable linker. The linker was varied from *n*-propylene (*n* = 3) to *n*-octylene (*n* = 8).^{85a,b} The observed stability constants (K_{obs}) with diboronic acid sensors 44 were generally larger than for monoboronic acid sensor 45. Stable cyclic 1:1 complexes are formed with glucose and galactose. Sensor 46 with a flexible six-carbon linker was selective for D-glucose ($K_{\text{obs}} = 962 \pm 70 \text{ dm}^{-3} \cdot \text{mol}^{-1}$). Interestingly, a switch to galactose selectivity is observed when the linker length increases, for example, *n*-heptylene (*n* = 7) and *n*-octylene (*n* = 8).

Modular diboronic acids with naphthylene, anthracene, and pyrene fluorophores and ridged *p*- or *m*-(diaminomethyl)benzyl linkers 47a–c and 48a–c were synthesized. These more rigid sensors were selective toward D-glucose and formed cyclic complexes with D-glucose as determined by circular dichroism (CD) spectroscopy. Interestingly, meta sensors 48a and 48b displayed particularly strong affinity for D-galactose, with K_{obs} of 330 and 299 $\text{dm}^{-3} \cdot \text{mol}^{-1}$ respectively.⁵³

It is worth pointing out that the basic design concept of the modular sensor system 43 has been further developed by Glysure Ltd. (www.glysure.com) into a fully functional continuous fiber-optic sensor 49 (cf. 46) for the continuous glucose monitoring of patients in intensive care units (ICU).

Scheme 17. Glucose-Specific Sensing by AIE-Active Bioprobe^a



^aReproduced with permission from ref 84. Copyright 2010 American Chemical Society.

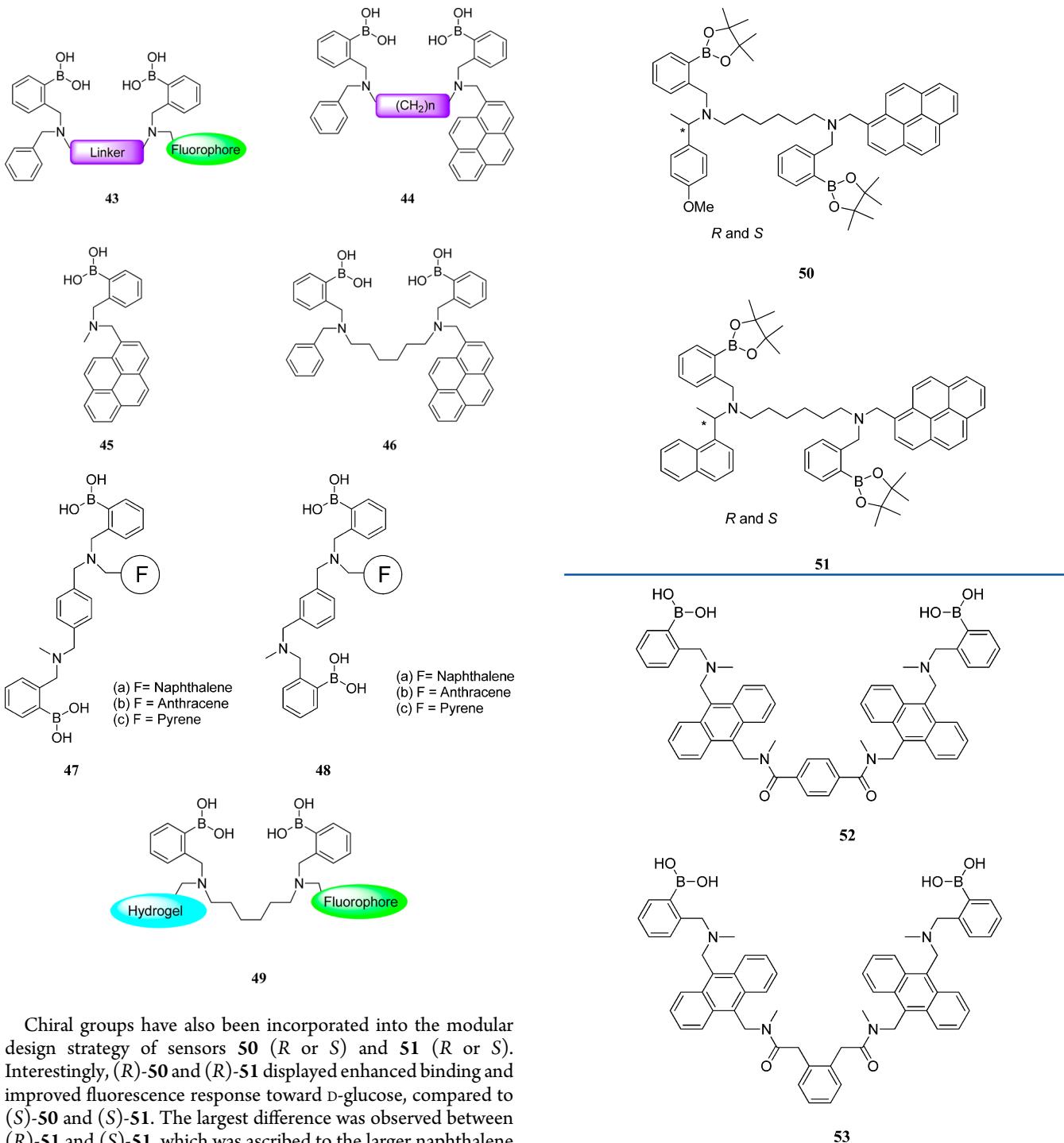
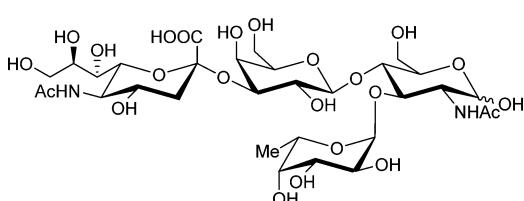


Figure 4. Diboronic acid sensors 52 and 53.

constant, the rigidity of the linker was lost, resulting in a 50% reduction of the observed stability constant (K_{obs}).

Chiral groups have also been incorporated into the modular design strategy of sensors **50** (*R* or *S*) and **51** (*R* or *S*). Interestingly, (*R*)-**50** and (*R*)-**51** displayed enhanced binding and improved fluorescence response toward D-glucose, compared to (*S*)-**50** and (*S*)-**51**. The largest difference was observed between (*R*)-**51** and (*S*)-**51**, which was ascribed to the larger naphthalene group resulting in enhanced steric factors. The chemoselectivity displayed by these chiral sensors has been explained by the better match of the binding pocket for the *R* chirality sensors and D-glucose. These observations clearly illustrate the importance of chirality, even in systems where chiral discrimination is not the goal.⁸⁹

Wang and co-workers⁸⁶ have prepared a series of diboronic acid sensors including **52** and **53**. A generic template based on the core of sensor **38** was used, but unlike many other systems, the spacing between the boronic acid groups is quite large (Figure 4). The sensor with a *p*-benzene linker **52** (Figure 4) was particularly selective for sialyl Lewis X **54**,^{86a,b} while sensor **53** with an *o*-xylene linker was selective for D-glucose.^{86c} Upon changing to a flexible butyl linker while the number of carbon atoms remained

**54.** sialyl Lewis X

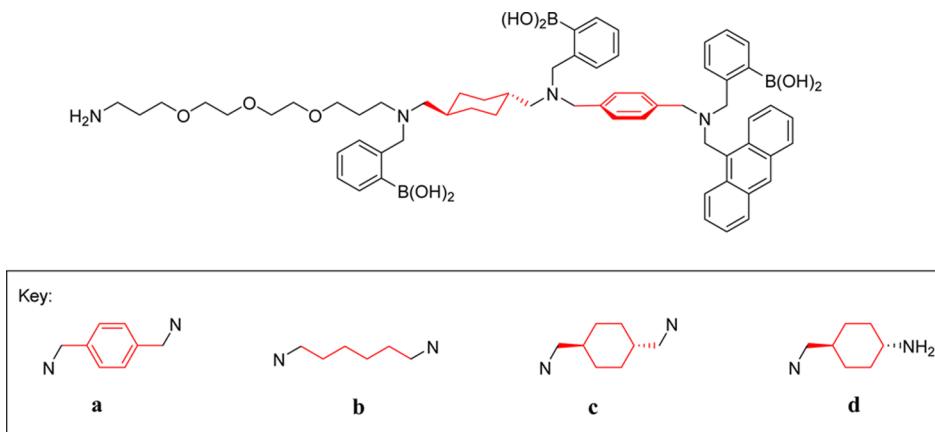


Figure 5. Sensors prepared by Hall and co-workers^{87a} (55) to evaluate three-point binding (variable linkers **a–d** are highlighted in red).

A solid-phase synthetic approach was used by Hall and co-workers^{87a} to prepare modular boronic acid based sensors. A variety of common components made it possible to assemble a library of compounds. The Hall approach produced structures where the interamine linkers could be altered (selectivity was again achieved for D-glucose with a six-carbon linker). Importantly, the approach evaluated a third boronic acid receptor unit for selective recognition of disaccharides (Figure 5).

Four disaccharides—lactulose, melibiose, turanose, and trehalose—were used to evaluate the triboronic acid sensors. Sadly, no advantage was found for systems with three (versus two) boronic acid receptor units: the observed stability constant (K_{obs}) for the triboronic acid sensors **55a** and **55c** with lactulose was 200 M^{-1} (Figure 5), which is similar to the value obtained for the diboronic acid sensor (with linker **a**), with a stability (K_{obs}) of 220 M^{-1} for lactulose at pH 7.8 in $0.010 \text{ mol}\cdot\text{dm}^{-3}$ (1:1 water/methanol) phosphate buffer.

Hall and co-workers^{87a} also performed a very important set of experiments to evaluate the electronic properties of the arylboronic acid receptors (**56**, Figure 6). By changing the Lewis acidity at boron, it was hoped that two attributes of the molecular recognition event could be changed: the strength of the saccharide binding interaction and the strength of the N–B interaction which controls the fluorescence signal intensity. The hypothesis proposed by Hall and co-workers was investigated by use of electron-withdrawing and electron-donating groups at the para position of the arylboronic acid.

The five groups investigated were methoxy, fluoro, methoxycarbonyl, cyano, and nitro, with para-substituent parameters, σ_p , of -0.12 , 0.15 , 0.44 , 0.70 , and 0.81 , respectively. If lactulose was used as a model disaccharide, a qualitative trend was observed in which electron-poor phenylboronic acids display enhanced binding. These observations were rationalized by the fact that, with increasing Lewis acidity at boron, the N–B interaction is enhanced, resulting in more tetrahedral character at boron. This in turn reduces ring strain in the boronic ester and reduces the pK_a of boron, allowing the sensor to work at a lower pH. In particular, methoxycarbonyl, cyano, and nitro groups appeared to be particularly effective at increasing the observed stability constants. The largest stability constant (K_{obs}) for lactulose was the diboronic acid with a *p*-cyano electron-withdrawing group (Figure 6).^{87a}

Hall and co-workers⁹⁰ have also taken a particularly interesting approach to enhance the binding strength of simple boronic acid units by using the enhanced binding of 4,6- or *cis*-3,4-diols with *o*-

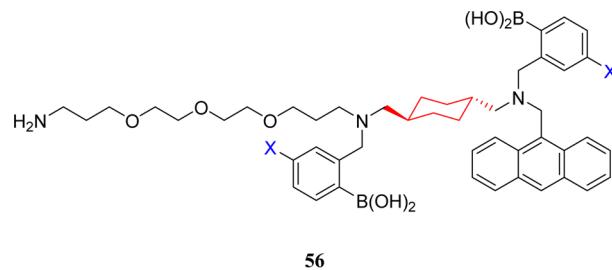
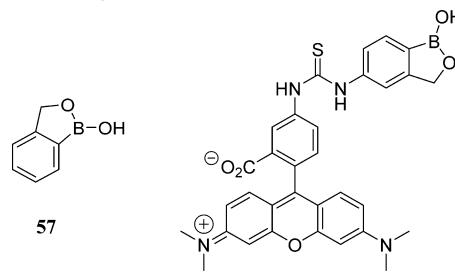
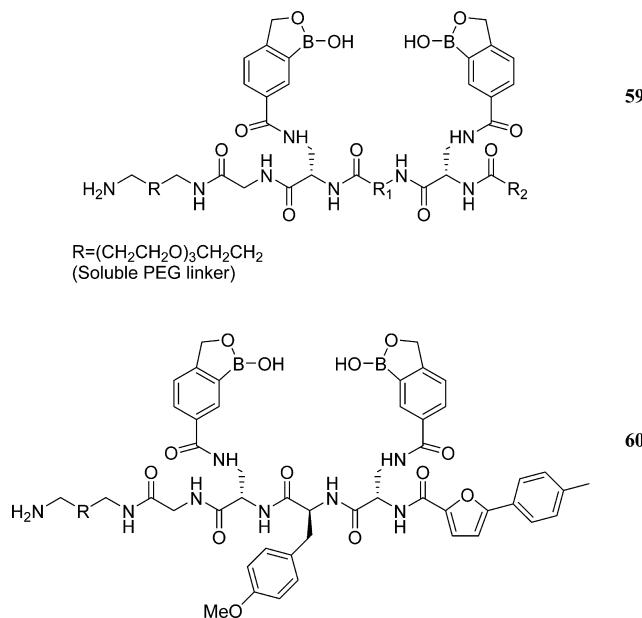


Figure 6. Sensors developed by Hall and co-workers^{87a} with variable linker (highlighted in red) and electron-withdrawing and electron-donating groups (highlighted in blue).

benzoboroxoles **57**. A UV assay with alizarin red S (ARS) in neutral water was used to determine the binding constants of *o*-benzoboroxole with glycopyranosides. The binding constant of *o*-benzoboroxole with D-glucose was larger ($K_{\text{obs}} = 22 \text{ M}^{-1}$) than that of phenylboronic acid with D-glucose ($K_{\text{obs}} = 5 \text{ M}^{-1}$). The importance of this unit for binding saccharides was rapidly recognized, and Hindsgaul and co-workers⁹¹ used the *o*-benzoboroxole unit attached to a tetramethylrhodamine dye **58** for the analysis of glycoproteins.

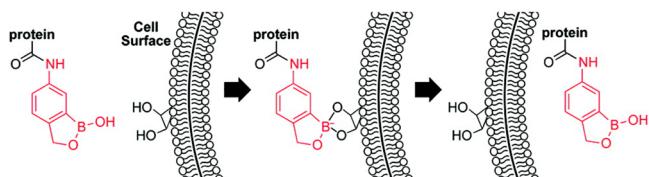


Hall and co-workers^{87b} have used the *o*-benzoboroxole receptor to produce a library of receptors targeted against the tumor-specific carbohydrate antigen Thomsen–Friedenreich (TF) disaccharide (Gal- β -1,3-GalNAc). Receptors **59** and **60** were constructed by use of peptide linkers to both simplify the synthesis and add additional hydrogen-bonding sites to the receptors. *o*-Benzoboroxoles were used rather than boronic acids because *o*-benzoboroxoles bind particularly well with 4,6- or *cis*-3,4-diols found in the TF antigen. By this approach, 400 receptors were prepared (varying R_1 and R_2), and a particularly potent receptor was discovered with an IC_{50} of $20 \mu\text{M}$.



Raines and co-workers⁹² have conjugated benzoxaborole moieties with bovine pancreatic ribonuclease (RNase A). 5-Amino-2-hydroxymethylphenylboronic acid was condensed with the protein carboxyl groups by use of a carbodiimide (Scheme 18). It was shown that the boronated protein has enhanced affinity

Scheme 18. Boron Receptor Conjugation and Proposed Mechanism of Cellular Delivery^a



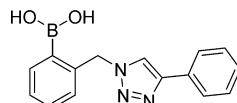
^aReproduced with permission from ref 92. Copyright 2012 American Chemical Society.

for oligosaccharides in vitro and also facilitates cellular uptake of the protein and enhances protein delivery to the cytosol. They concluded that such boronates have many attractive attributes and could be used as mediators in drug delivery.

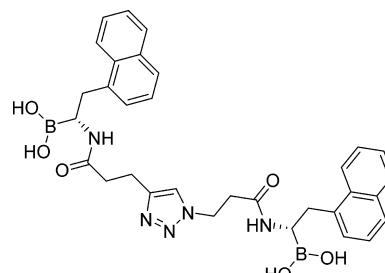
Anslyn and co-workers⁹³ have strongly advocated peptide-based boronic acid receptors for pattern-based saccharide sensing. Array systems have been used to identify the saccharide sucralose in a complex beverage sample. Similarly, peptide-based boronic acid systems have been evaluated by Lavigne and co-workers,⁹⁴ who have developed bead based boronolectins and used the receptors to investigate glycoproteins and oligosaccharides with fluorescent labels, while Duggan and Offermann⁹⁵ have investigated polymer bound receptors derived from 4-borono-L-phenylalanine.

A click-fluor compound, **61**, has been developed by use of the Huisgen [3 + 2] cycloaddition to generate a fluorescent boronic acid;⁹⁶ this click approach is well-suited to modular sensor development. The click reaction was used to prepare a 1,2,3-triazole from an azide and a terminal alkyne, resulting in a fluorescent sensor using nonfluorescent building blocks. An important aspect of these click-fluor sensors is that many triazole fluorophores can be generated due to diverse availability of acetylene units. There is some doubt surrounding the original selectivity order reported for the click-fluor, and this is currently

under reevaluation by Fossey.⁹⁷ This does not detract from the fact that the click-fluor concept is particularly well-suited to sensor array development.⁹⁸



Wang and co-workers⁹⁹ have prepared water-soluble fluorescent amidoboronic acids that display similar binding properties to standard boronic acids. They have also prepared diboronic acid **62** by use of click chemistry. The diboronic acids display enhanced binding for glucose, fructose, and sorbitol and display significant binding for maltose and lactose.¹⁰⁰



Anslyn and Sessler and co-workers¹⁰¹ have developed a diboronic acid porphyrin receptor for ginsenoside detection. The fluorescence of the receptor is quenched by the 1:1 bound ginsenoside guests. The ginsenoside saccharide units are bound to the boronic acid groups, while the steroid core and porphyrin ring participate in hydrophobic interactions.

Boronic acid–saccharide interactions were also employed for the fluorescence visualization of tumors. A sensor system prepared from a peptide connected with two boronic acid groups exhibited high specificity toward sialyl Lewis X and was used for selective labeling of cell-surface glycans of human hepatic cancer cells.¹⁰² Overexpressed saccharide-based carcinoma biomarker sialyl Lewis X was selectively labeled in the mouse xenograft tumor via this novel boronolectin fluorophore¹⁰³ (Figure 7).

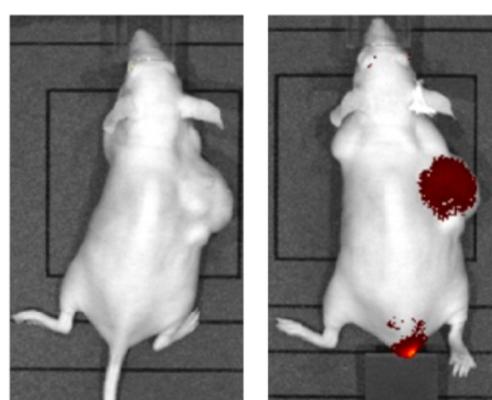
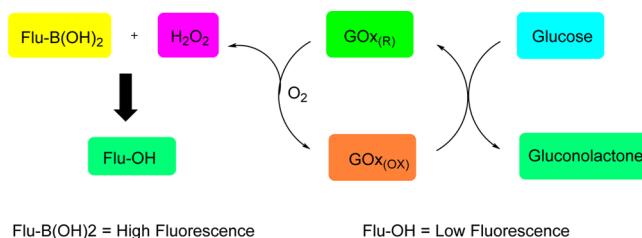


Figure 7. Optical imaging of xenograft tumor boronolectin fluorophore: (left) mouse before imaging agent injection; (right) mouse 24 h after tail vein injection of the contrast agent, showing almost exclusive delivery to the tumor site. Reproduced with permission from ref 103. Copyright 2013 Elsevier.

An interesting combined chemo/biosensor has been developed by Tomapatanaget and co-workers,¹⁰⁴ who have used the generation of hydrogen peroxide from the enzymatic oxidation of glucose by glucose oxidase (GOx) to generate a fluorescence response (Scheme 19). A fluorescent boronic acid is oxidized by

Scheme 19. GOx Enzymatic System for Glucose Detection by Use of Boronic-Based Fluorescent Sensors

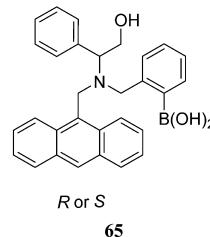
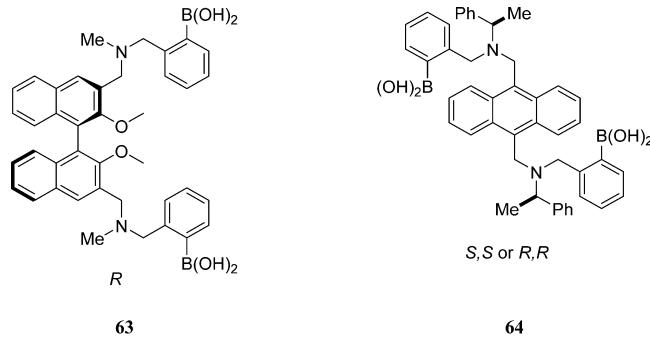


hydrogen peroxide to give a nonfluorescent phenol. Thus, the concentration of glucose can be monitored indirectly by hydrogen peroxide-mediated oxidation of the boronic acid fluorophore.

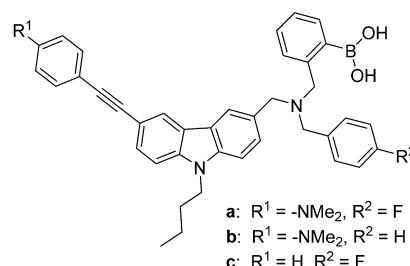
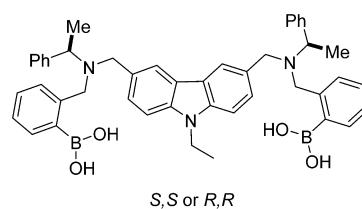
4.2. Chirality and Its Importance in Developing Saccharide-Selective Systems

Although saccharides are available in nature in a single chiral form, the chirality of a synthetic receptor can be used to either enhance or reduce the binding with a particular saccharide. The enhanced binding of *R* chirality modular sensors, described in the previous section, clearly demonstrates the importance of chiral units in the design of selective saccharide receptors.⁸⁹ Therefore, while the use of chiral units for glucose-selective receptors has to date been limited, we believe that chiral units are very important in the design of saccharide-selective sensors with practical utility. Hence we will spend some time covering the development of boronic acid-based chiral receptors for species other than glucose, since the knowledge derived from these receptors can also be applied to the development of glucose-selective receptors.

The development of chiral fluorescent boronic acid sensors has been the focus of interest for a number of years^{72,89,105} and has recently been reviewed.¹⁰⁶ In 1995 the first chiral fluorescent boronic acid sensor for saccharides was developed. The 1,1'-binaphthalene-2,2'-diol (BINOL) moiety performs the roles of fluorophore, scaffold, and stereogenic center. Gray and Houston¹⁰⁷ found that the racemate of sensor **63** bound sugar acids such as tartaric acid with a similar strength to monosaccharides. Subsequently, enantioselective recognition of sugar acids (such as tartaric acids) by use of enantiopure ligands was investigated by Zhao et al.^{105c} However, BINOL is a UV fluorophore, and longer-wavelength visible emissions are desirable in the development of practical systems. Furthermore, with the BINOL-based chiral sensors the fluorophore and the stereogenic centers are fixed. Therefore, with these sensors it is difficult to change both the fluorophore and binding site independently, which is required in order to optimize the molecular sensing performance of these systems. Several of the limitations with the BINOL-based systems were overcome with chiral boronic acid sensors developed with anthracene; these sensors produced long-wavelength emission and good chiral selectivity toward tartaric acid, other sugar acids, and sugar alcohols (sensor **64**). The monoboronic acid system *R*-**65** and *S*-**65** was also evaluated and found to be enantioselective for mono-*a*-hydroxylcarboxylic acids, including mandelic acid and lactic acid.¹⁰⁸

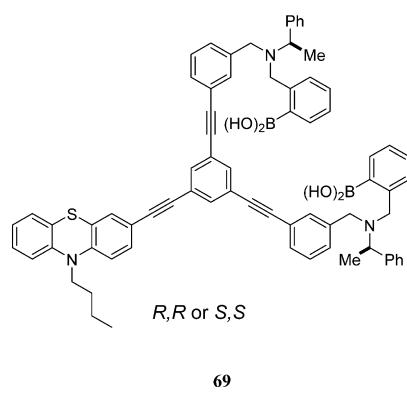
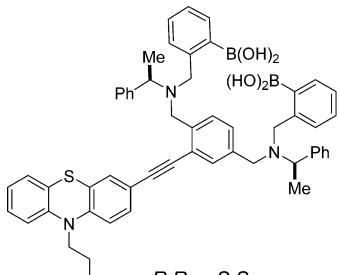


Chiral carbazole boronic acids **66** and **67** were constructed, where the fluorophore is the electron donor and the protonated amine/boronic acid moiety is the acceptor of a d-PET system. Importantly, emission for the sensor at acidic pH is lower than with normal PET sensors where the fluorophore is the acceptor (*a*-PET).^{105f,h} However, use of an integrated carbazole fluorophore in these sensors restricts systematic modification of the molecular scaffold.



A major disadvantage with the BINOL, anthracene, and carbazole systems is that the fluorophore, scaffold, and chirogenic centers are a single entity (sensors **63**, **64**, and **66**), which means that the choices available for the fluorophore used in these chiral sensors is restricted. Also, with carbazole as the fluorophore, PET efficiency of the d-PET boronic acid sensors is particularly low: the emission enhancement (or contrast ratio) is about 2.0-fold. However, the *a*-PET sensor **64** has a much larger contrast ratio of around 10-fold.

In order to separate the fluorophore, scaffold, and chirogenic centers, sensors **68** and **69** were designed, where the fluorophore (phenothiazine) and the chirogenic centers [(*R*)- or (*S*)-*α*-benzylamine] are joined to the scaffold (2-iodo-1,4-benzenediacarboxaldehyde).^{72b} Use of a rigid linker between the fluorophore and the boronic acid receptors ensures any interactions between the binding sites and the fluorophore are minimized. Phenothiazine was selected as the d-PET fluorophore because the chromophore is a strong electron donor. A contrast ratio of 8-fold was achieved with the new sensors, which is much better than the carbazole-based d-PET fluorescent sensors.^{72a,105b,i} Significant enantioselective discrimination between D- and L-tartaric acid was obtained by use of these sensors. The affinity of the sensors with different analytes varied and depended on the size of the boronic acid binding pocket. The sensors could also be used for chemoselective discrimination of disaccharides (sucrose, lactose, and maltose) and glycosylated steroids (ginsenosides).



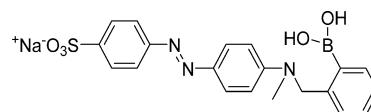
4.3. Colorimetric Sensing Applications

Sensors for saccharides that produce a colorimetric response are of particular interest in a practical sense: if a system can produce a large color change, then it could be used to help develop a diagnostic test paper for saccharides, comparable to universal indicator paper used to determine pH. This type of sensor would allow D-glucose concentrations to be measured without the need of specialist instrumentation. Such systems would be of considerable benefit to diabetics in developing countries.

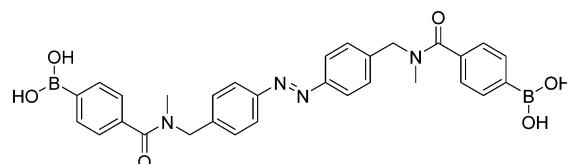
Boronic acid azo dyes have been used for many years in the treatment of cancer by a technique called boron neutron capture therapy (BNCT). However, it was not until the 1990s that related dyes were investigated for saccharide binding. In particular, Nagasaki et al.¹⁰⁹ investigated diazo chromophores with boronic acid groups (which aggregate in water); upon binding with saccharides, the system deaggregated and changed color. The observed behavior is due to saccharide complexation causing an increase in the hydrophilicity of the complex. A boronic acid-

based diazo dye was developed by Takeuchi et al.,¹¹⁰ which produces a change in the absorption band upon the addition of nucleosides.

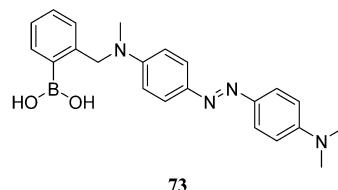
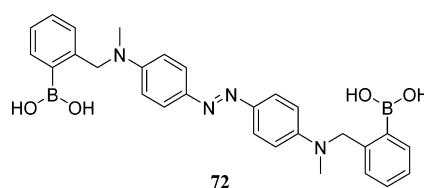
The internal charge transfer (ICT) sensor **70**, developed by Sandanayake and Shinkai,¹¹¹ uses an intramolecular interaction between the neighboring amine and the boronic acid unit to produce color changes upon the addition of saccharides. The electron-rich amine creates a basic environment close to the electron-deficient boron atom, which enhances the interaction between boronic acid and saccharide and reduces the working pH of the sensor. The electronic changes caused by this reduction in the pK_a of the boronic acid upon saccharide binding causes a spectral change in the attached ICT chromophore and is detected as a color change. The observed stability constant for D-fructose at pH 7.6 in water (K_{obs}) with **70** was 138 M^{-1} . However, a binding constant with D-glucose could not be established due to negligible spectral changes.



Shinmori et al.¹¹² developed a light-gated saccharide sensor **71** containing an azobenzene unit that is isomerized from the stable *trans* conformation to the thermodynamically unfavorable *cis* isomer by photoirradiation; the system has high D-glucose and D-allose selectivity. This is because a stable cyclic 1:1 complex is formed between the *cis*-azoboronic acid dye and these saccharides.

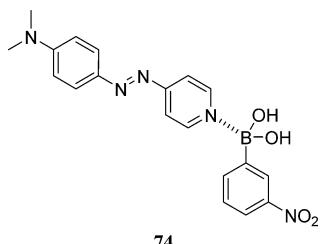


Koumoto and Shinkai¹¹³ evaluated azobenzene derivatives **72** and **73**, bearing one or two aminomethylphenylboronic acid groups, for colorimetric sensing of saccharides in aqueous media. The observed stability constants for D-fructose (K_{obs}) with **73** was 433 M^{-1} and that for D-glucose was 13.0 M^{-1} in 1:1 (v/v) methanol/water at pH 7.5 (phosphate buffer).

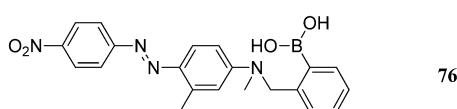
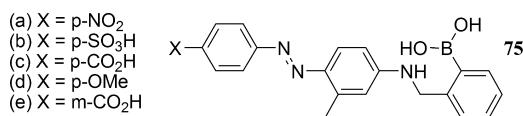


The boronic acid–amine interaction has been judiciously employed by Koumoto et al.¹¹⁴ for the sensing of saccharides. The

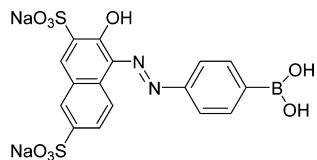
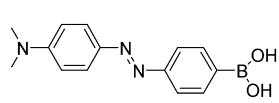
boron of 3-nitrophenylboronic acid interacts with the pyridine nitrogen of 4-(4-dimethylaminophenylazo)pyridine **74** in methanol, and the color changes from yellow to orange. When the boronic acid group binds with saccharides, the acidity of the boronic acid group is increased, resulting in a stronger boron–nitrogen interaction that enhances the intramolecular charge-transfer band and causes the solution to turn red.



Our group has prepared a diazoboronic acid system **75a** that produces a large naked-eye color change from purple to red upon saccharide binding.¹¹⁵ With azo dye **75a**, upon saccharide binding the wavelength maximum shifts to shorter wavelength (ca. 55 nm).



For diazoboronic acid **70** at intermediate pH, an N–B interaction exists; however, at high and low pH the interaction is broken. With dye molecule **75a**, the presence of an anilinic hydrogen makes the system particularly interesting, since it can result in different species at high pH. In the absence of saccharide, at pH 11.32, **75a** is purple, but with added saccharide the color becomes red. This is because the N–B interaction in the presence of saccharides becomes stronger. This increased N–B interaction makes the N–H proton more acidic. Thus, at pH 11.32, the saccharide–boronate complex forms the red species with N–B bond via loss of a water molecule (H⁺ from aniline and OH⁻ from boronate). These observations explain why sensor **70** does not produce a visible color change upon saccharide binding. For sensor **70** there is no possibility of dehydration; therefore no spectral shift is observed. This explanation has been confirmed by use of sensor **76**, which does not have a N–H. In the case of **76**, upon addition of saccharides no change in color was observed.^{115b} Evaluation of a series of azo dyes with electron-donating and electron-withdrawing groups, **75a–e**, confirms that a strong electron-withdrawing group is necessary to produce a visible color change.^{115b}

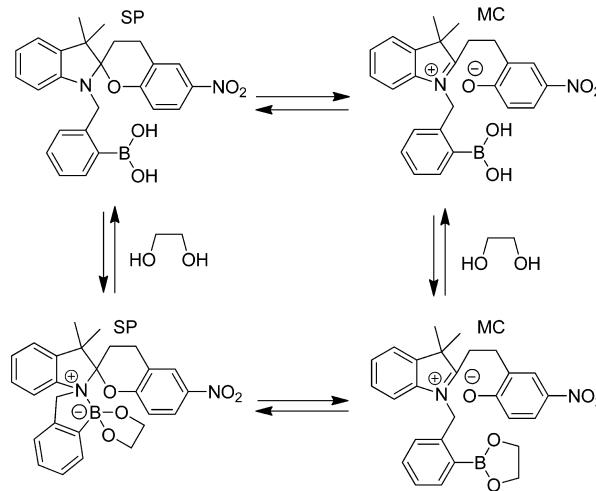


DiCesare and Lakowicz¹¹⁶ have developed boronic acid azo dyes **77** and **78**, in which direct conjugation with the boron center

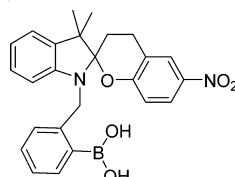
is possible; in particular, azo dye **78** produces a visible color change from yellow to orange at pH 7.

The spirobenzopyran boronic acid **79** has been evaluated by Shinmori et al.¹¹⁷ and undergoes changes in absorption spectra upon addition of saccharides. The addition of saccharides to **79** alters the balance of the merocyanine (MC) to spiropyran (SP) equilibrium and thus alters the color of the system. Upon addition

Scheme 20. Spiropyran versus Merocyanine Equilibrium with Saccharides



of saccharides to **79**, the SP species is preferred because of the formation of a stronger N–B interaction (Scheme 20).

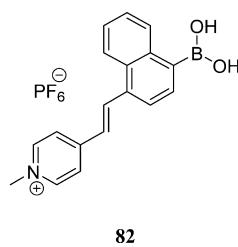
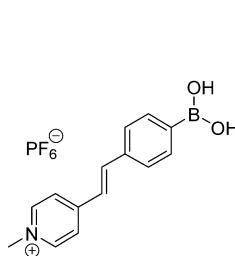
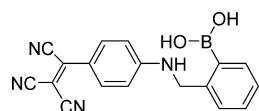


79

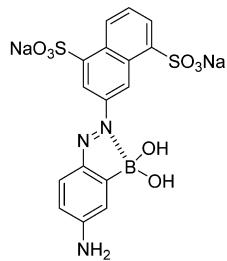
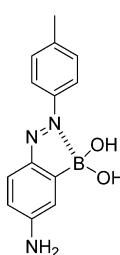
A tetraboronic acid resorcinarene system has been developed by Strongin and co-workers¹¹⁸ for visual sensing of saccharides. Saccharides produce characteristic color changes on gentle heating in dimethyl sulfoxide (DMSO). Strongin and co-workers¹¹⁹ have shown that “oxygen” and the resorcinol hydroxyl groups are key to the color changes observed. Xanthenes formed in situ by heating resorcinols in DMSO have been implicated in the observed color changes. Non-boronic acid receptors also produce colored solutions but to a much lesser extent. With non-boronic acid systems, the color is due to hydrogen bonding between aldonic acids (heating sugars in DMSO produces aldonic acid derivatives) and the xanthene chromophore.^{118b,119}

Our group was inspired by the results obtained with **75a–e**, and hence we evaluated the strongly electron-withdrawing tricyanovinyl dye **80**. The pK_a of **80** (7.81) was much less than that of azo dye **75** (10.2), which means that a visible color change could be observed at pH 8.21.¹²⁰

Sato et al.¹²¹ developed stilbazoliumboronic acids **81** and **82** and then evaluated these units for saccharide sensing. Wang and co-workers¹²² have used nitrophenolboronic acids, which show large UV shifts upon addition of saccharides.



A clever system has been developed by Egawa et al.,¹²³ who have shown that saccharide binding with *o*-azoboronic dyes **83** and **84** causes a change in color due to an N–B interaction. The *o*-azoboronic acids produce significant changes in the UV–vis absorption spectra upon saccharide binding. The *o*-azoboronic acids have also been evaluated attached to a poly(ethylenimine) polymer.¹²⁴ Egawa et al.¹²⁵ have also prepared an excellent review of colorimetric sugar sensing by use of boronic acid-substituted azobenzenes.



A novel dye displacement assay has been developed by Tsuchiya and Kanekiyo,¹²⁶ using the inclusion complex of boronic acid-modified amylose with iodine, which is bluish-purple. Upon addition of polyhydroxy compounds, the color of the solution fades, due to the dissociation of iodine from the amylose cavity in response to binding of the polyhydroxyl compounds with the boronic acid groups. It was postulated that electrostatic repulsion between the anionic boronate groups and iodine drives the colorimetric response.

The boronic acid–fructose interaction has been followed during a fermentation process via changes in pH. Changes in color of rhodamine B dye were used to monitor the pH and produce a colorimetric output.¹²⁷ Kanekiyo and co-workers¹²⁸ have used a boronic acid-containing polycation to prepare a pattern-printed microscope slide, by a layer-by-layer approach, to prepare a colorimetric saccharide-sensing chip. Menon and co-workers¹²⁹ have elegantly employed gold nanoparticles (AuNPs) functionalized with glucose-selective calix[4]arene/phenyldiboronic acid for colorimetric analysis of glucose concentrations as low as 4.3 nM in human blood serum samples.

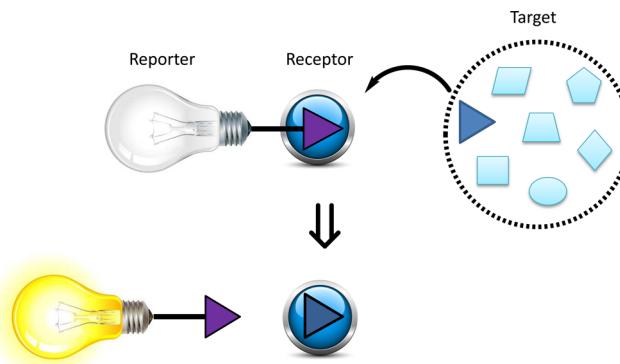
Chang and co-workers¹³⁰ have used seven boronic acid derivatives and 39 pH indicators to generate a colorimetric array consisting of 273 indicator pairs. Subsequently, 11 of the best pairs were selected for dose-dependent evaluation by principal component analysis (PCA), and the array was used to analyze serial concentrations of glucose, fructose, and sucrose.

The array could be applied to quantify the sugar content in saline solutions. Suslick and co-workers¹³¹ have prepared a system for the identification of sweeteners, and Musto and Suslick^{45b} have written a review on colorimetric arrays.

4.4. Displacement Assays for Sensing Applications

The preceding sections cover the use of boronic acids in the development of integrated molecular sensors. These integrated systems consist of a receptor and reporter (fluorophore or chromophore) as part of a single molecular unit. There is, however, another approach that can be used to develop boronic acid-based sensors: a competitive assay. A competitive assay uses a receptor and reporter (typically a commercial dye) that associate under the measurement conditions. The receptor–reporter complex formed can then be selectively dissociated by addition of an appropriate guest. A sensor system results because reporter dissociation from the receptor results in a measurable response (Scheme 21).

Scheme 21. Cartoon Outlining the Utility of an Assay System^a

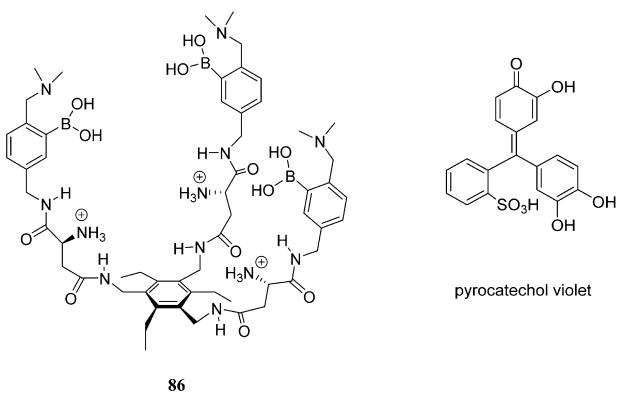
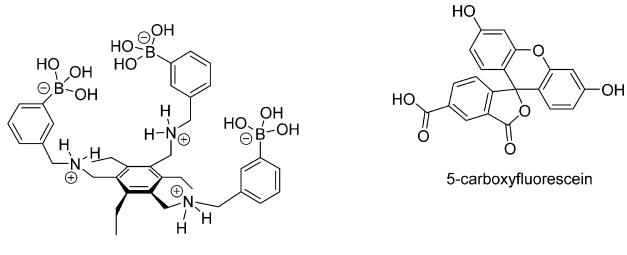


^aPrepared by use of light bulb (Kraska/Shutterstock) and blue button images (Roman Sotola/Shutterstock).

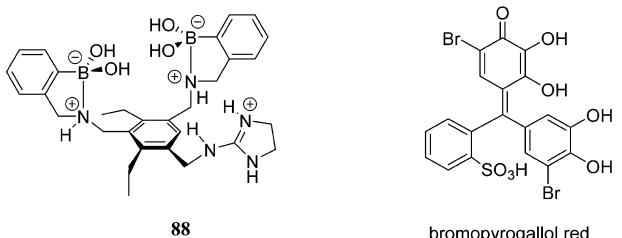
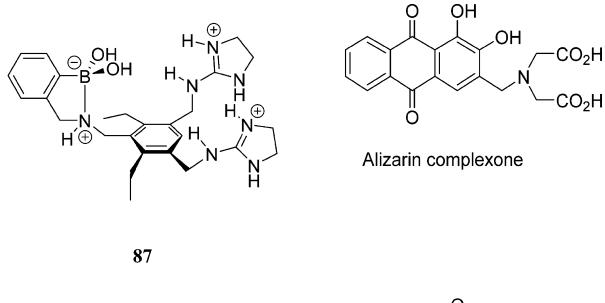
Dye displacement assays are primarily competitive binding systems where an analyte displaces a dye from the receptor, and the displacement of the dye results in a change in color. The colorimetric (or fluorescence) changes of the dye are then used to determine the amount of analyte present. Pioneering and seminal research in this area include the influential protocols of Anslyn and co-workers,¹³² Buryak and Severin,¹³³ and Singaram and co-workers,¹³⁴ which are excellent examples of supramolecular sensing.

Two very elegant systems that use boronic acid receptors have been published by Anslyn and co-workers. In particular, the C₃ symmetric tripodal boronic acid **85** has been developed as a D-glucose 6-phosphate-selective receptor.¹³⁶ Binding of D-glucose 6-phosphate was measured by the competitive displacement of 5-carboxyfluorescein. The addition of D-glucose 6-phosphate causes a decrease in the 494 nm absorption band, allowing the concentration of the guest to be directly monitored. More elaborate tripodal C₃ symmetric boronic acid receptors have also been prepared by Anslyn. Heparin binding with receptor **86**, is detected by the displacement of pyrocatechol violet.¹³⁷

For sensor **87**, the competitive displacement of alizarin complexone can be used to measure the binding of tartrate or malate anions. In a particularly interesting report, sensor system **87** has also been used to evaluate malate in pinot noir grapes.¹³⁸ The authenticity of whiskies could be determined by use of receptor **88** and pyrocatechol violet in an assay for gallic acids.



This is a nice assay since an increase in the concentration of gallic acid is strongly correlated with the vintage and hence value of the whiskies.¹³⁹ A combination of **87** and **88** and pyrocatechol violet and bromopyrogallol red indicators have been used to quantify tartrate and malate.¹⁴⁰ Pyrocatechol violet and **88** have been used to evaluate the reaction kinetics for the formation of tartaric acid produced via dihydroxylation of malic acid.¹⁴¹ Anslyn and co-workers¹⁴² have also very elegantly coupled indicators with chiral boronic acids to prepare enantioselective assay systems for diols and α -hydroxyl carboxylates.

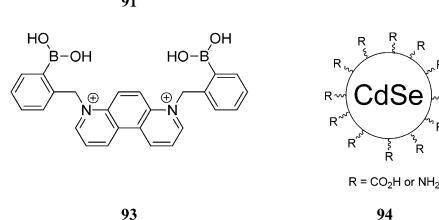
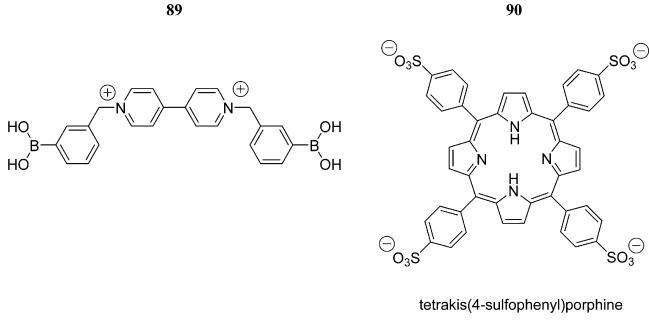
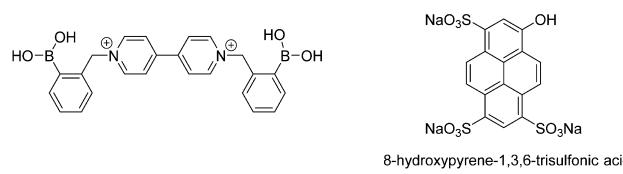


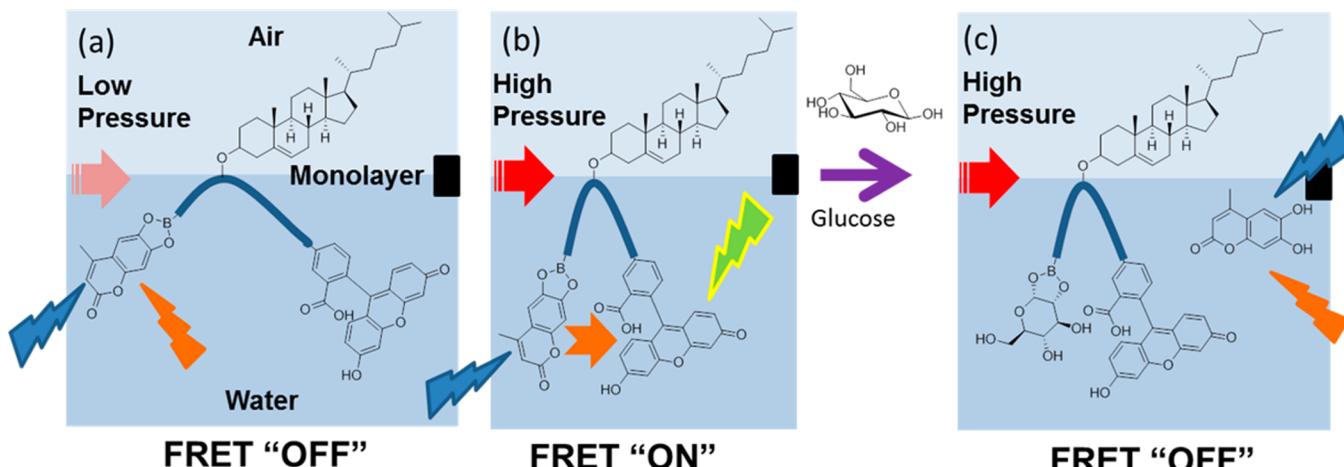
A very nice system developed by Anslyn and co-workers¹⁴³ is the detection and classification of ginsenosides and ginseng by use of boronic acid receptors and dynamic multicomponent indicator displacement sensor arrays. Anslyn and Ariga and co-workers¹⁴⁴ have recently developed an exciting mechanically controlled displacement assay for glucose (Scheme 22).

Lakowicz and co-workers¹⁴⁵ have developed competitive systems that use a ruthenium metal–ligand complex, a boronic acid derivative and D-glucose. A reversible complex between 2-

tolylboronic acid or 2-methoxyphenyl boronic acid and the metal–ligand complex is formed. Formation of the complex results in a 7-fold increase in luminescent intensity. Subsequent addition of saccharides such as D-glucose to the ruthenium complex reduces the luminescent intensity, due to a decreased in the binding between the metal–ligand complex and the boronic acid. It is important to note that ruthenium complexes are particularly useful in the development of practical optical sensing systems because they can be used for lifetime-based sensing applications.

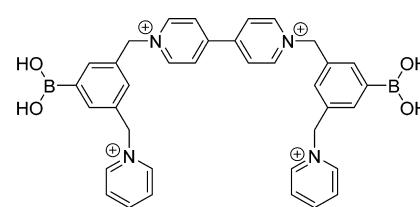
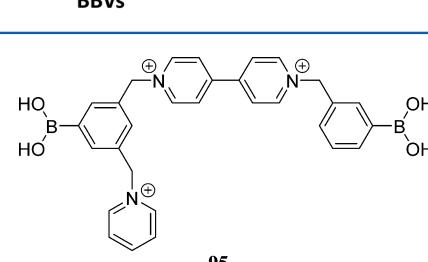
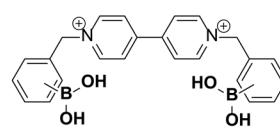
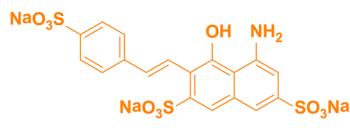
Singaram and co-workers¹⁴⁶ have developed a very interesting multicomponent system that uses the quenching of negatively charged (anionic) pyranine dye **90** by positively charged diboronic acid viologen units **89**, **91**, and **93** to detect saccharides. Viologen **89** binds well with D-fructose ($K_{obs} = 2600 \text{ M}^{-1}$) and weakly with D-glucose ($K_{obs} = 43 \text{ M}^{-1}$) in pH 7.4 phosphate buffer,^{146a} while viologen **91** also binds weakly with D-glucose^{146c}, and only 4–6% fluorescence recovery is produced by both systems. However, viologen **93** binds well with both D-fructose ($K_{obs} = 3300 \text{ M}^{-1}$) and D-glucose ($K_{obs} = 1800 \text{ M}^{-1}$) in pH 7.4 phosphate buffer. Importantly, as well as enhanced selectivity for D-glucose, a large 45% fluorescence recovery was determined upon saccharide addition.^{146b} Viologen **91** and tetrakis(4-sulfophenyl)porphine fluorophore **92** also produce a large 33% fluorescence recovery upon addition of saccharides, with a binding constant for D-glucose of 14 M^{-1} .^{134e} From a device development perspective, it is important to note that viologen **89** has been used successfully to detect saccharides with fluorescent quantum dots (FQD) **94**. FQD are important materials because they are bright and have good photostability with broad absorption and narrow emission bands.^{88c} Given that fluorescent recovery on D-glucose binding for the quantum dot viologen system was reasonable, the system has the potential to be developed into a practical sensor system. The importance of this type of system is clear, given that the binding of viologen **89** with FQD has also been used for detection of glucose by Feng et al.¹⁴⁷



Scheme 22. Mechanically Controlled Displacement Assay^a

^aFluorescence spectral change of the monolayer at the air–water interface upon compression (a → b) and upon the addition of glucose (b → c) is depicted.

Scheme 23. BBV/Dye Structure and Sensing Mechanism for Detection of D-Glucose



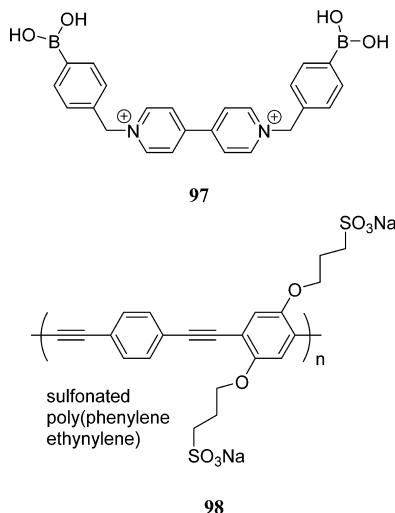
Similarly, Feng and co-workers¹⁴⁸ have designed and constructed three systems composed of fluorescent dye (NAHBDS) and boronic acid-substituted viologen (BBV) quenchers/receptors for sensing D-glucose in pH 7.4 buffer solution (Scheme 23).

Viologens 95 and 96 were used by Singaram and co-workers¹⁴⁹ to quantify the effect of increasing the number of positive charges on the viologen unit 91 (i.e., 2, 3, and 4). It was found that viologen units with more positive charges resulted in enhanced quenching of the negatively charged (anionic) dye; that is, interaction of the negatively charged dyes with more positively charged viologens is larger. However, since dissociation of the viologen–dye complex becomes more difficult, D-glucose sensing ability is reduced. Singaram and co-workers¹⁵⁰ evaluated the effect of varying the negatively charged (anionic) dye component with viologen 96, using 11 different negatively charged anionic dye molecules, and found that sensor systems for saccharides worked better with more highly charged anionic dyes.

Quenching and recovery of a sulfonated poly(phenylene ethynylene) by a positively charged diboronic acid viologen 97 upon the addition of saccharides has been reported by Lakowicz and co-workers.¹⁵¹ The system developed was D-fructose-selective and resulted in 70-fold fluorescence enhancement upon addition of saccharides.

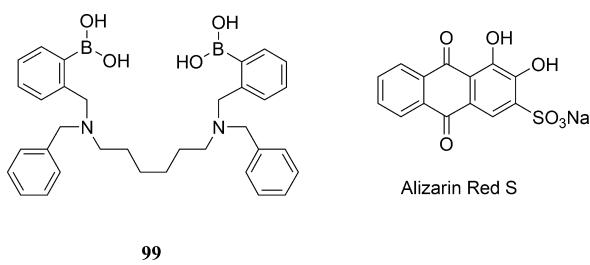
Wang and co-workers¹⁵² have used alizarin red S and phenylboronic acid (PBA) in a competitive assay for saccharides.

The indicator displacement assay system formed between ARS and PBA is D-fructose-selective.⁵¹ The system uses the well-known interaction of alizarin red S with boronic acids.¹⁵³ The stability constant (K_{obs}) determined for D-fructose (160 M^{-1}) was larger than that obtained for D-glucose (4.6 M^{-1}) in water at pH 7.4 (phosphate buffer). In extending this elegant system, Hu and co-workers¹⁵⁴ used 3-pyridinylboronic acid in an assay for D-glucose with pyrocatechol violet as the dye. The observed stability



constant (K_{obs}) in water at pH 7.4 (phosphate buffer) for D-glucose was 272 M^{-1} . The most important aspect of these indicator displacement assay systems is that they are very simple and yet such systems are capable of detecting millimolar D-glucose concentrations. From a practical angle, the fluorescence response of these indicator displacement assay systems can be improved by use of surfactants (e.g., cetyltrimethylammonium bromide, CTAB), since the fluorescence intensity can be enhanced in the hydrophobic core of micelles.¹⁵⁵

A D-glucose-selective fluorescent indicator displacement assay has been developed that uses alizarin red S.¹⁵⁶ The boronic acid sensor 99 and alizarin red S displays a 6-fold enhancement in binding for D-glucose over the simple PBA system. Sensor 99 can also be used at 10 times lower concentrations than the simple PBA system. The observed stability constants (K_{obs}) for 99 in 52.1 wt % methanol/water at pH 8.21 (phosphate buffer) were 140 M^{-1} for D-fructose and 66 M^{-1} for D-glucose.



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Basu and co-workers¹⁵⁷ have used alizarin red S and commercially available monoboronic acids and determined that 3-methoxycarbonyl-5-nitrophenylboronic acid was superior to PBA in indicator displacement assays. A temperature-sensitive copolymer containing boronic acid units was used by Elmas et al.,¹⁵⁸ and loss of fluorescence due to displacement of ARS upon exposure to a series of analytes was observed.

Hydrogel spheres containing boronic acid receptors were used in an ARS displacement assay. By use of these hydrogel spheres, the relative amounts of saccharides in fruit juices could be estimated.¹⁵⁹ Poly(lactic acid) (PLA)-containing boronic acids have been developed as potential drug delivery conjugates.¹⁶⁰ Boronic acid-terminated PLA (BA-PLA) was prepared and the formation of the well-defined fluorescent ARS-PLA conjugates was used to follow the binding of a range of hydroxyl-containing species. In the system ARS was selectively displaced by diols. We envision that the simple BA-PLA system could be developed into versatile and highly specific polymer-drug conjugates and

nanoconjugates, which are important targets for drug delivery vehicles.

Boron polymers have been reviewed by Jäkle¹⁶¹ and are of great interest in various scenarios including electronic, optical, and sensing applications. Modular fluorescent receptors have been incorporated into polymer constructs,^{85a} and hydrogels have found application in indicator displacement assays: for example, Singaram and co-workers^{88d,146c,162} demonstrated a polyacrylamide boron pyridinium conjugate (100, 101, Figure 8).

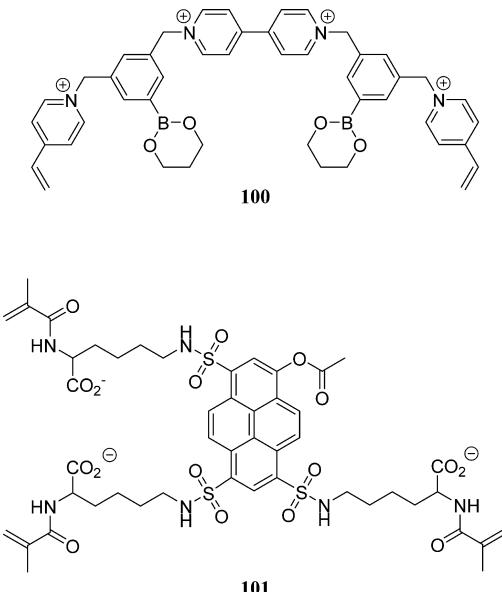


Figure 8. Building blocks for hydrogel boronic acid sensors of Singaram and co-workers.

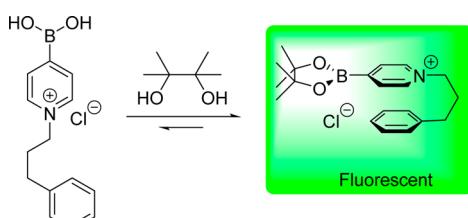
Fluorescent hydrogels have been polymerized directly into multiwell plates at ambient temperature and analyzed with a plate reader, in order to produce sensors for measuring pH and glucose concentrations.¹⁶³

Zhang and co-workers¹⁶⁴ have also used the interaction of diboronic acid viologens 89, 91, and 97 to produce fructose-selective systems. Feng and Wang and co-workers¹⁶⁵ have followed in the footsteps of Singaram and used boronic acid-functionalized benzyl viologen 89 with a BINOL-based water-soluble conjugated polymer. The two-component system is glucose-sensitive, producing a 17-fold fluorescence increase in the presence of 100 mM glucose. Feng and co-workers¹⁶⁶ have used a cationic polymeric viologen boronic acid and anionic dye to develop a glucose-specific fluorescence sensor. The two-component probe showed a reversible fluorescence on-off response with glucose.

4.5. Cation-π Interactions for Sensing Applications

Our research has shown that pyridinium cation-π interactions can be measured by fluorescence spectroscopy.¹⁶⁷ Thus, the combination of cation-π interactions and pyridiniumboronic acids into a single unit can be used to develop fluorescence saccharide sensors. A propylene (Leonard linker)-connected phenylalkylpyridiniumboronic acid produces cation-π stacking-induced exciplex fluorescence (102, Scheme 24).¹⁶⁸ The system was then evaluated with anions, and the largest fluorescence response was observed with the boronic acid ion pair with diffuse negative charge (e.g., boronic acid, $X^- = \text{PF}_6^-$). The relative fluorescence intensity trend ($\text{PF}_6^- > \text{Br}^- > \text{Cl}^- > \text{F}^-$) clearly

Scheme 24. Phenylpropylpyridium Boronic Acid Receptor for Diols



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demonstrates that tighter ion-pair interactions result in weaker fluorescence intensity, so for a turn-on sensor it is better to start with low fluorescence (tight pair), since under these conditions a larger fluorescence increase with added diol guest is possible. Thus, when $X = \text{PF}_6^-$ the fluorescence does not change; that is, the maximum fluorescence intensity has been achieved. Therefore, the largest fluorescence turn-on was obtained for pinacol when chloride was used for the counteranion ($X = \text{Cl}^-$).

4.6. Fluorophore Quencher Interactions for Sensing Applications

Singaram and co-workers^{88,134,146,149,150,162,169} have exquisitely coupled a fluorophore and viologen into systems for diol and saccharide detection. James and Fossey have suggested that the molecular-beacon fluorophore and quencher pairs methodology used in quantitative PCR assays such as Taqman could be employed to measure boron–diol interactions.¹⁷⁰

The concept of the fluorophore–quencher sensing system is shown in Figure 9. A fluorescein boronic acid was used as the

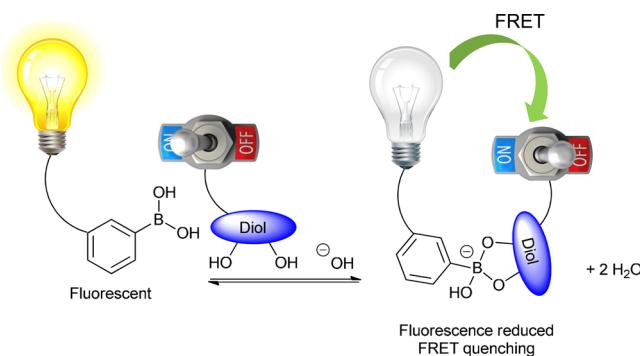


Figure 9. Boronic acid fluorophore interacting with a diol quencher. Prepared by use of light bulb (Kraska/Shutterstock) and toggle switch images (Gl0ck/Shutterstock).

fluorophore and a series of diols based on methyl red were synthesized as the quencher partners, used to probe a fluorescence resonance energy transfer (FRET) quenching/sensing system based on boronate ester formation (see Figure 10).¹⁷⁰ Use of a fluorescein boronic acid and diol-appended quencher combination **103a–c** indicated that boronate ester formation does indeed result in improved quenching, with **103c** producing the best quenching.¹⁷⁰

The well-known and elegant alizarin red S (ARS)–phenylboronic acid displacement assay developed by Springsteen and Wang^{152a} was adapted to use a fluorescent dye and a quencher, boronic acid-appended viologen. The quenched fluorescence of the dye was recovered upon boronic acid–glucose interaction.¹⁴⁸

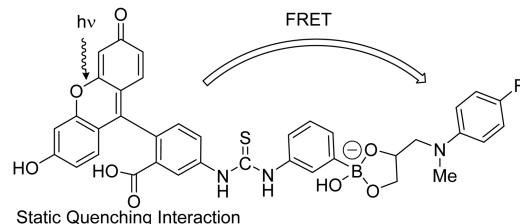
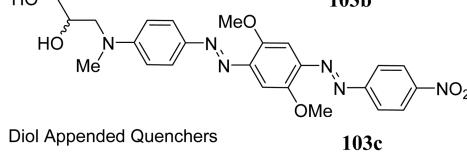
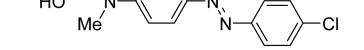
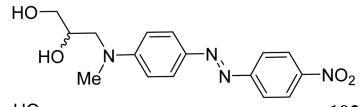
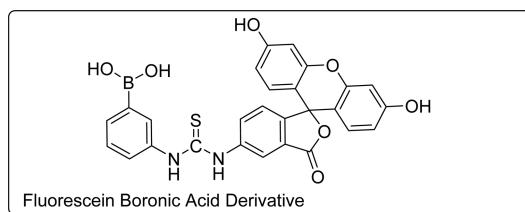
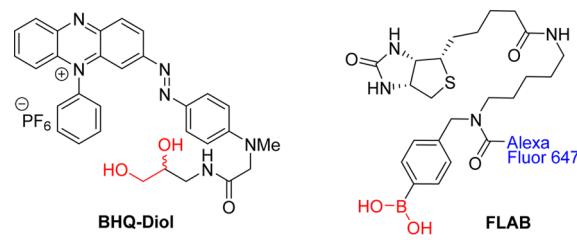


Figure 10. Fluorescein boronic acid derivative, diol appended quenchers, and cartoon showing quenching by FRET.

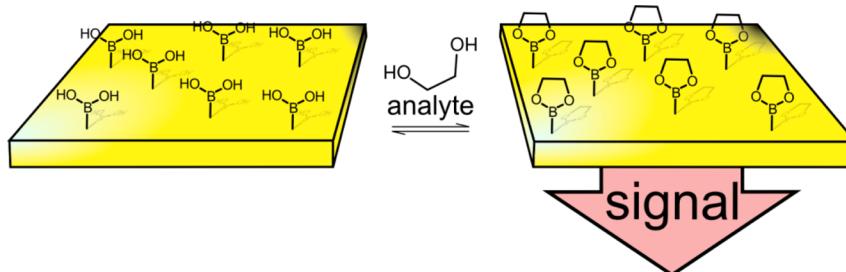
4.7. Surfaces for Sensing Applications

Advantageous sensing can be achieved by the attachment of sensor molecules to a surface (Scheme 25). The sensing format is heterogeneous because the binding and detection event take place at the liquid/solid interface. Such systems are desirable since they can be quickly developed into “real” analytical systems.

Although fluorescence is more often used for homogeneous systems, it can also be used for heterogeneous systems. As an example a sensor was prepared at the gold-streptavidin surface using a fluorophore linked to boronic acid and biotin (FLAB, **105**). The sensor used biotin for surface attachment to streptavidin, a boronic acid receptor and a fluorophore (Alexa-fluor 647, Invitrogen, $\text{Ex}_{\text{max}} 647 \text{ nm}$). The quencher-diol complex was constructed using a quencher for Alexa-Fluor 647, BHQ-3 (Biosearch Tech).¹⁷¹ The binding of a FLAB to the streptavidin coated gold surface was confirmed by both surface plasmon resonance (SPR) and fluorescence (f-SPR). Upon treatment of the surface with BHQ-diol **104**, both an SPR response and fluorescence quenching were observed.



Scheme 25. Heterogeneous Detection of Diols by Means of Boronic Acid-Based Sensors Assembled in Monolayers on a Simple Surface^a

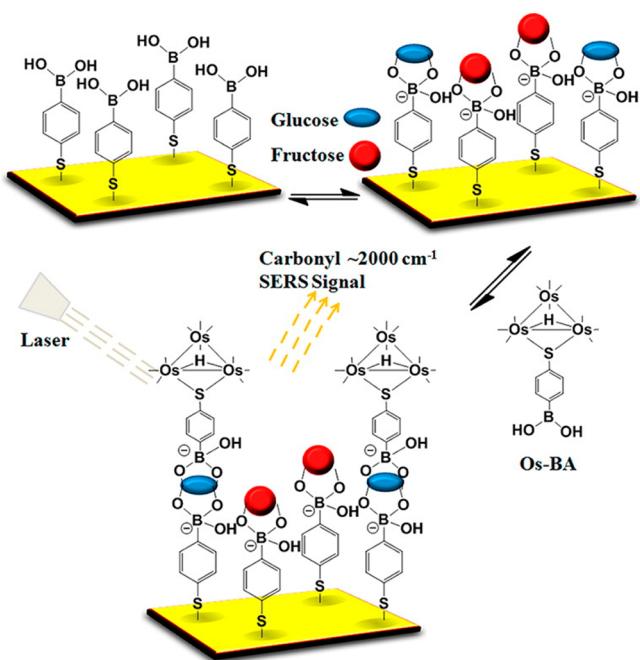


^aReproduced with permission from ref 45r. Copyright 2014 Springer Verlag.

FLAB fluorescein was also used to coat SuperAvidin polystyrene microspheres (Bang Laboratories) as seen by fluorescence microscopy.¹⁷² Such spheres, it was hoped, could be used to develop sensor arrays.

A D-glucose-selective SPR sensor **106** was developed by use of a glucose-selective diboronic acid unit with thiolic functionality, allowing simple attachment to a gold surface.¹⁷³ A simple boronic acid was used for a surface-enhanced Raman scattering (SERS) monosaccharide assay (Scheme 26). The boronic acid served as capturing agent, and the triosmium carbonylboronic acid was employed as a secondary label (in a sandwich assay).¹⁷⁴

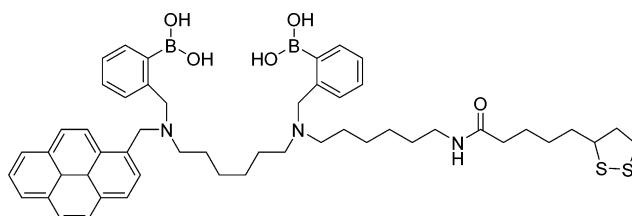
Scheme 26. Boronic Acid-Based SERS Sensors for Monosaccharides^a



^aSelectivity for glucose is achieved with 2:1 sandwichlike binding. Reproduced with permission from ref 174. Copyright 2013 American Chemical Society.

A recent multicolorimetric sensor array was developed that used boronic acid-based thin films combined with three anionic dyes for colorimetric saccharide detection.¹⁷⁵

Hindsgaul and co-workers⁹¹ have developed a simple naked-eye method to determine terminal glycosylation of a glycoprotein (Scheme 27). The colorimetric method uses the release of terminal galactose (Gal) by treatment of a glycoprotein with β -



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galactosidase. Gal is captured on hydroxylaminated glass beads, forming a red fluorescent boronic acid and Gal complex, with the color of the beads quantifying the amount of captured Gal.

Strano and co-workers¹⁷⁶ have employed a series of phenylboronic acids conjugated to a polymer of poly(ethylene glycol) in order to disperse single-walled carbon nanotubes (SWNTs). Saccharide addition quenches the near-infrared fluorescence (Figure 11). Strano and co-workers¹⁷⁷ have also screened a library of 30 boronic acids with sodium cholate-suspended SWNTs for their ability to reversibly report glucose binding via a change in SWNT fluorescence (Scheme 28). It was demonstrated that the BA-SWNT complex fluorescence intensity modulates with glucose over a physiologically important concentration range (5–30 mM).

James and He and co-workers¹⁷⁸ have crafted simple materials for sensing monosaccharides by using graphene oxide (GO) with boronate-based fluorescence probes (**107** and **108**). The fluorescence of the probes is quenched by GO via FRET. However, upon addition of saccharides (fructose), the fluorescence of the composite BA@GO sensor is switched on (Scheme 29).

Minami et al.¹⁷⁹ have developed a particularly promising organic field effect transistor (OFET) functionalized by a phenylboronic acid monolayer for the detection of saccharides. By use of this OFET device, glucose concentrations >5 mM could be detected, making the system suitable for the measurement of plasma glucose levels.

4.8. Polymers for Sensing Applications

A significant enhancement of binding, and hence the performance of a sensor system, can be achieved by incorporation of a boronic acid unit into a polymer matrix (Scheme 30). Inclusion of the molecular sensor in the polymer can help in the development of superior analytical devices, since the polymer imparts many advantages such as improved robustness, sensitivity, handling, and biocompatibility. These properties are vital for the development of noninvasive D-glucose sensors.

Scheme 27. Visual Detection of Terminal Glycosylation State of a Glycoprotein

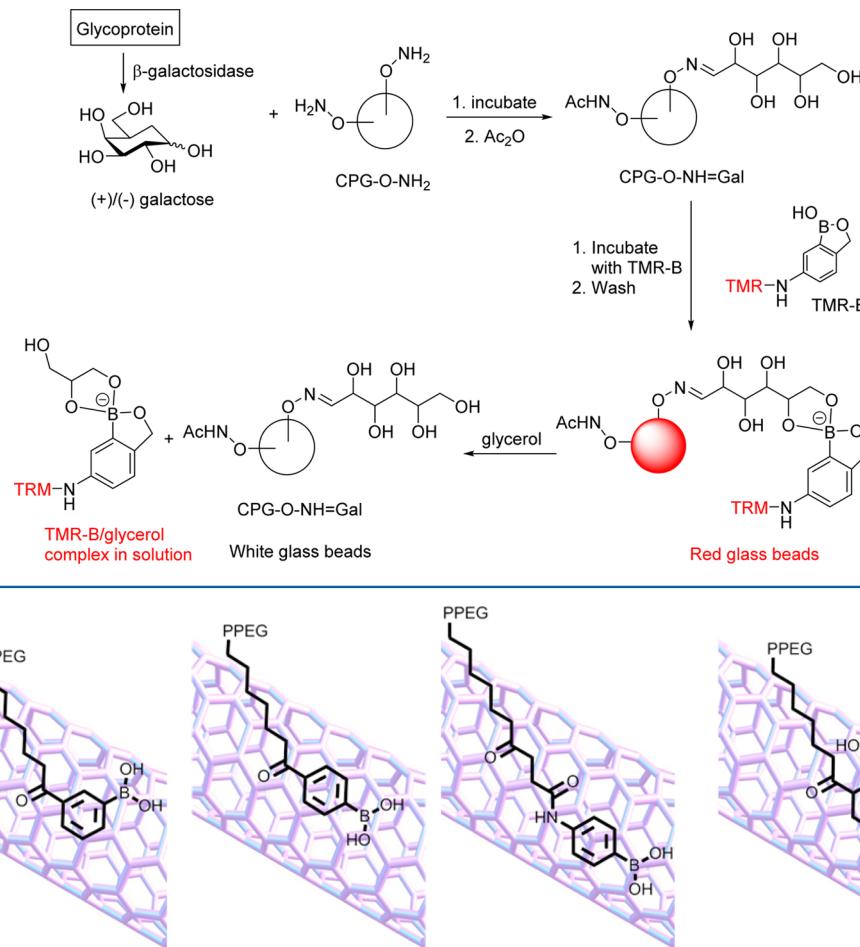
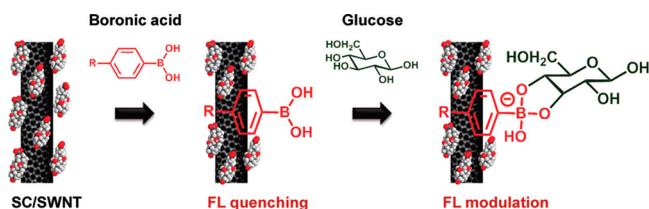


Figure 11. Phenylboronic acids conjugated with a poly(ethylene glycol) polymer and used to disperse single-walled carbon nanotubes (SWNTs). Prepared by use of carbon nanotube image (foxterrier2005/Shutterstock).

Scheme 28. Boronic Acid Derivatives Form Complexes with Sodium Cholate-Suspended SWNTs and Report Glucose Binding via Changes in SWNT Fluorescence^a

^aReproduced with permission from ref 177. Copyright 2011 American Chemical Society.

Functional boronic acid-based polymers can be traced back to the seminal work of Wulff.¹⁸⁰ Wulff developed aromatic boronic acids having *o*-dialkylaminomethyl groups, and these boronic acids exhibit a lower pK_a value due to the intramolecular N–B interaction, resulting in stable boronate esters at neutral pH. Some of the most influential work on polymer hydrogel sensors has been carried out by Asher and co-workers,¹⁸¹ who have pioneered photonic crystal carbohydrate sensors and Lowe and co-workers,¹⁸² who have explored the use of glucose-selective holographic sensors.

Photonic crystals consisting of a copolymer of acrylamide and styrene gel scaffold with tethered boronic acid were developed to

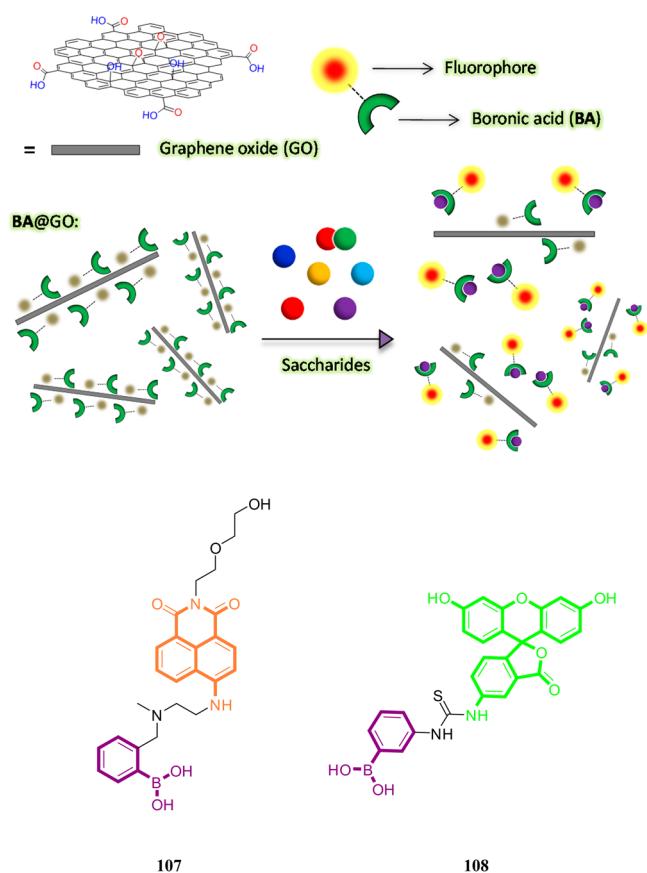
be used for determination of glucose in tears by use of contact lenses.¹⁸³ Gold nanoparticles functionalized with rhodamine B and boronic acid moieties were shown to be suitable for the detection of glucose in tear fluid. The method relies on the revival of quenched fluorescence in the presence of glucose.¹⁸⁴

Zhou and co-workers¹⁸⁵ have shown that CdS quantum dots immobilized in boronic acid-based microgels can be used for fluorescence detection of glucose. Glucose-sensitive microgels capable of measuring physiologically important glucose concentration (1–25 mM) were prepared.

Zhou and co-workers¹⁸⁶ have also described the construction of multifunctional hybrid nanogels. The system was designed for the simultaneous optical detection of glucose and self-regulated insulin release at physiological pH and temperature (Figure 12). These hybrid nanogels are composed of silver nanoparticle (AgNP) cores coated by a copolymer gel shell of poly[4-vinylphenylboronic acid-*co*-2-(dimethylamino)ethyl acrylate]. Inclusion of a glucose-sensitive gel shell onto the AgNPs makes the polymer-bound AgNPs glucose-responsive.

Zhou and co-workers¹⁸⁷ have used the modification of CdTe/ZnTe/ZnS core/shell/shell (CSS) quantum dots (QDs) with phenylboronic acid to create PBA-modified QD probes (PBA-QDs) which can be used to detect and quantify glucose in cells (Scheme 31). The unique glucose-mediated assembly of PBA-QDs could be used to modulate the photoluminescence

Scheme 29. Fluorogenic BA@GO Sensors for Selective Detection of Monosaccharides^a



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properties of the QDs and enables a selective ratiometric glucose response.

Yu and Yam¹⁸⁸ have developed a glucose-responsive fluorescence polymer. The glucose complex formed with the boronic acid groups of the polymer backbone causes the polymer to become negatively charged (boronate anion formation), which in turn causes aggregation of the positively charged trimethyl-pentylammoniumpyrene that had been added to the polymer. Thus, glucose can produce a ratiometric fluorescence response as the excimer emission increases. Kim et al.¹⁸⁹ have developed a glucose-selective fluorescent system based on hyperbranched

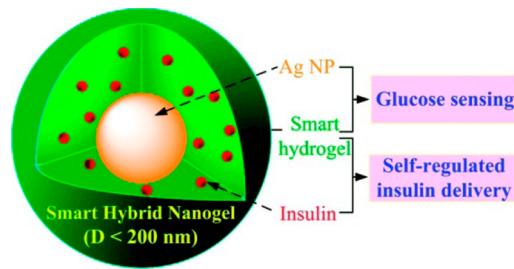
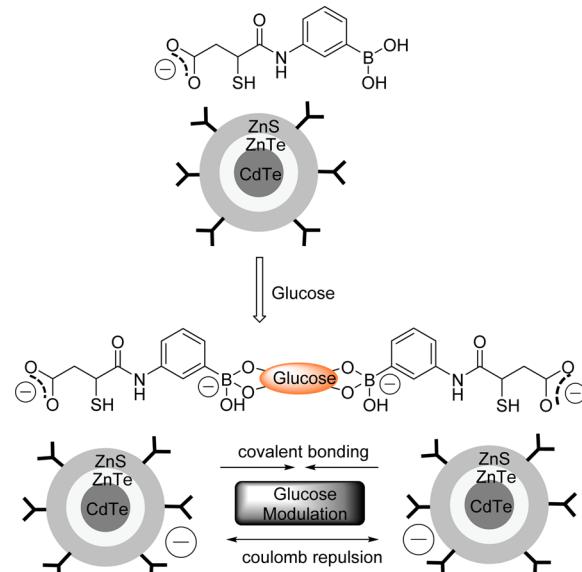


Figure 12. Smart hybrid nanogels that can integrate optical glucose detection and self-regulated insulin delivery at physiological pH and temperature. Reproduced with permission from ref 186. Copyright 2010 American Chemical Society.

Scheme 31. Glucose-Mediated Assembly of PBA-Modified CdTe/ZnTe/ZnS CSS Quantum Dots^a



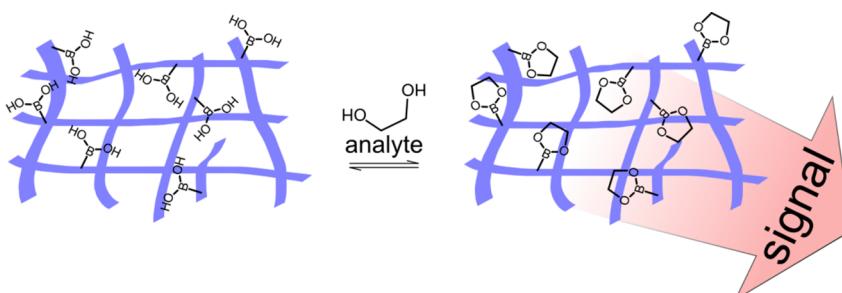
^aFluorescence of the QDs depends on binding between the analyte and molecular-recognition groups anchored to the surface of the QDs.

poly(*p*-phenylene) with boronic acid end groups capable of interacting with saccharides.

Takeuchi and co-workers¹⁹⁰ have developed a very interesting system by incorporating the known glucose receptor 38 into injectable hydrogel microbeads for continuous fluorescence glucose monitoring *in vivo* (Figure 13).

Subsequently, they switched to using fibers, which have some advantages over microbeads for long-term glucose monitoring in

Scheme 30. Detection of Diols by Use of Boronic Acid-Appended Polymer Matrix^a



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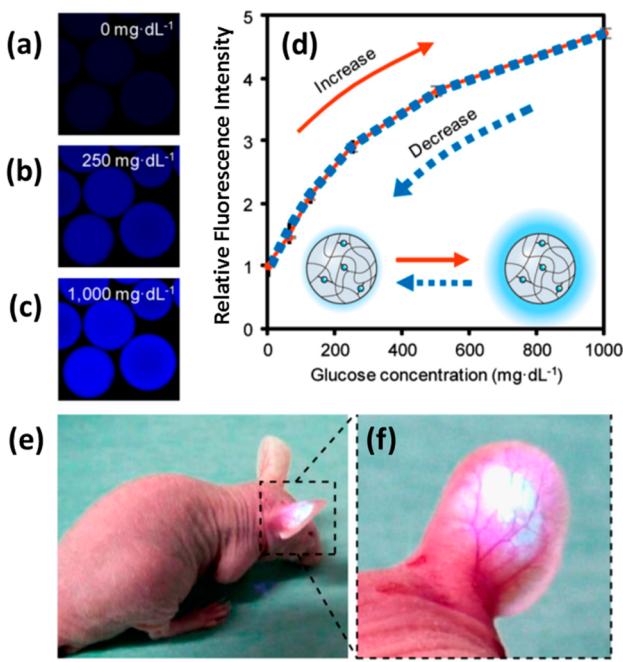


Figure 13. Fluorescent images of glucose-responsive fluorescence beads at glucose concentrations of (a) 0, (b) 250, and (c) 1000 mg·dL⁻¹. (d) Fluorescence intensity of beads with an increase and decrease in glucose concentrations. (e, f) Glucose-responsive fluorescence beads under the dermis of a mouse ear. Reproduced with permission from ref 190. Copyright 2010 National Academy of Sciences.

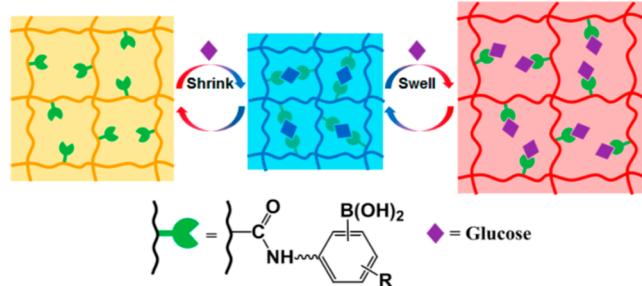
vivo: (i) fibers can remain at the implantation site for an extended period, whereas microbeads tend to disperse; (ii) fibers can be implanted with controllable and quantifiable fluorescence by simply cutting them to a particular length, thereby enabling stable and repeatable sensor functionality; and (iii) fibers can be readily and nonsurgically removed from the body. While the material is biocompatible, it is much better to remove the fibers after use to ensure that potential side effects are minimized¹⁹¹ (Scheme 32).

A sensor consisting of a polyacrylamide-based matrix with three different fluorescent probes for oxygen, glucose, and one reference channel was used to detect the consumption of glucose and oxygen in bacterial and mammalian cells.¹⁹² The D-glucose-

sensitive probe was based on a diboronic acid,⁷¹ and oxygen sensing used a modified perfluoro platinum porphyrin probe.

Changes in the gel diameter (swelling or shrinking) can be used for detection and also for therapeutic purposes. An acrylamide gel containing boronic acid and low-cost fluorescent dye Bordeaux R was used for the detection of glucose. Bordeaux R interacts with amide groups within the gel and causes the gel to shrink. Upon exposure of the gel to D-glucose, the polymer network swells and distorts the π–π stacking in assemblies of dye molecules embedded in the gel.¹⁹³ The therapeutic application can be exemplified via a poly(acrylic–boronic acid) gel, which shrinks or swells depending on the D-glucose concentration (Scheme 33).

Scheme 33. Concentration-Dependent Shrinking or Swelling of Polymer^a

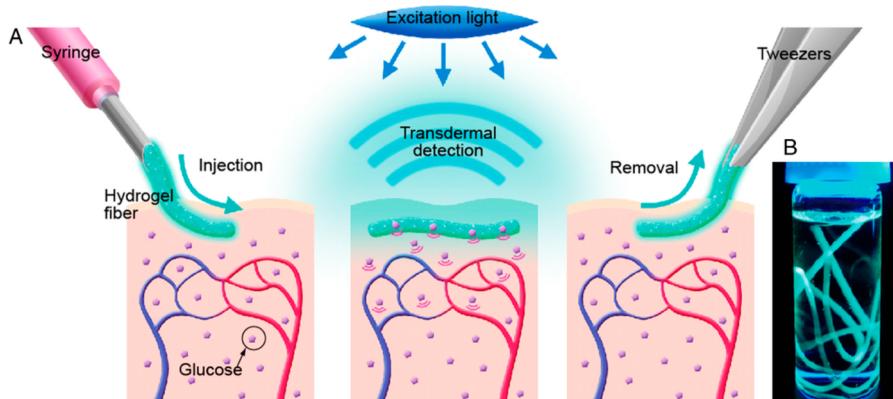


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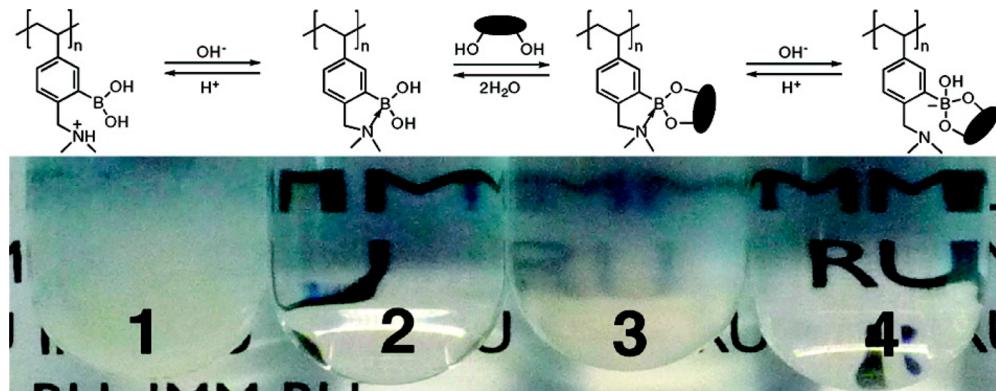
Such systems could be used for the D-glucose-controlled release of insulin.¹⁹⁴ Matsumoto and co-workers¹⁹⁵ have developed hydrogels for glucose sensing. Subsequently, Matsumoto and Miyahara and co-workers¹⁹⁶ have developed hydrogels for both continuous glucose intravascular sensors and the development of self-regulated insulin delivery systems.

Shi and co-workers¹⁹⁷ have used the self-assembly of phenylboronic acid-containing block copolymer and a glycopolymers to create glucose-responsive micelles. These micelles have the potential to be used as glucose-responsive drug delivery systems. Yang and co-workers¹⁹⁸ have developed block polymers

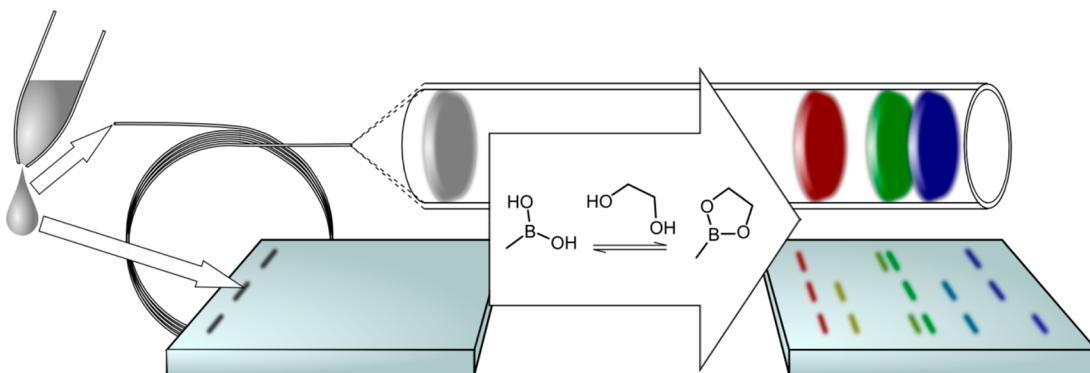
Scheme 32. (A) Illustration of Fluorescent Hydrogel Fiber Designed for Long-Term In Vivo Glucose Monitoring^a and (B) Fluorescent Hydrogel Fibers in 50% Glucose Solution, Excited by UV Light^b



^aThe fiber can be injected into subcutaneous tissues. The implanted fiber remains at the implantation site for a long period and transmits fluorescent signals depending on blood glucose concentration. The implanted fiber can be easily removed from the implantation site after use. ^bReproduced with permission from ref 191. Copyright 2011 National Academy of Sciences.

Scheme 34. Tubes Containing Polymer in Different Buffers after 18 h^a

^aTube 1, PBS (pH = 7.4); tube 2, PBS/D-fructose (50 mM); tube 3, PBS/D-glucose (100 mM); tube 4, Tris/D-glucose (100 mM, pH = 7.8). Reproduced with permission from ref 207. Copyright 2009 American Chemical Society.

Scheme 35. Utilization of Specific Boronic Acid–Diol Interactions for Capillary or Gel Electrophoresis^a

^aReproduced with permission from ref 45r. Copyright 2014 Springer Verlag.

containing a phenylborate ester, which can self-assemble to form nanoparticles and responded to glucose concentration changes at neutral pH and have the potential for use in self-regulated delivery of insulin.

Liu and co-workers¹⁹⁹ have prepared microgels containing a boronic acid receptor and two dye molecules (benzoxadiazole donor and rhodamine B acceptor) as a FRET pair. The efficiency of the FRET donors and acceptors in the microgels could be modified via thermally induced microgel collapse or glucose-induced microgel swelling at suitable pH and temperatures. The ratiometric fluorescent glucose sensor at 37 °C has significantly enhanced sensitivity over that observed at room temperature (25 °C).

Copolymerization of tertiary amines (Lewis bases) into glucose-responsive hydrogels with boronic acid groups results in a zwitterionic hydrogel with an enhanced selectivity for glucose relative to fructose, and the hydrogel shrinks (rather than swells) in response to increases in the concentration of glucose. This property had been attributed to the formation of reversible cross-links in the hydrogels with glucose. However, Horkay and Magda and co-workers²⁰⁰ have shown that changes in the thermodynamic mixing interactions between polymer network and solvent are the most important factors controlling swelling and shrinking of the hydrogels.

The viscosity of a polyacrylamide scaffold was used for analytical purposes. Changes in viscosity due to fluctuations in D-glucose concentrations were detected by a microelectromechanical system (MEMS).²⁰¹ A Sepharose gel with appended

boronic acid functionality (click chemistry) exhibited dose-dependent fluorescence intensity upon binding with D-glucose and D-fructose.²⁰² The fluorophore ARS was used to monitor the binding properties of amphiphilic boronic acid vesicles in a detection system for diols.²⁰³ Electropolymerization is a technique for tightly controlled and defined preparation of polymeric structures. A copolymer of phenylboronic acid with thiophene was used for potentiometric detection of D-glucose.²⁰⁴

Many different materials and methods have been used for the preparation of diol sensors based on molecularly imprinted polymers (MIP). The boronic acid unit, which is a small molecule with specific receptor abilities, is extremely suitable for MIP technology. Acrylate-based MIP immobilized on a QCM (quartz crystal microbalance) sensor were used for selective recognition of immunoglobulin M (IgM) and mannose over fructose.²⁰⁵ MIP technology was also used for preparation of photonic crystal technology with sensitivity towards glucose.²⁰⁶

van Hest and co-workers²⁰⁷ have used Wulff-type styrenic monomers and polymerization by radical addition–fragmentation chain transfer (RAFT). The resultant boronic acid polymers bind with saccharides due to the presence of the intramolecular N–B interaction, which lowers the pK_a of the boronic acid. Binding of the polymer with saccharides is observed via solubilization of the polymer upon complex formation. The polymer remained insoluble in phosphate-buffered saline (PBS) at pH 7.4 (tube 1) until D-fructose (50 mM) was added (tube 2). However, addition of D-glucose (100 mM) did not solubilize the

polymer in PBS (tube 3), but the polymer was completely soluble in a Tris/HCl buffer at pH 7.8 (tube 4) (Scheme 34).

4.9. Capillary or Gel Electrophoresis

Boronic acid is well suited for affinity-based detection and separation techniques such as electrophoresis (Scheme 35). Electrophoresis uses polyacrylamide hydrogel polymers for the separation of molecules on the basis of their size and charge. The approach is used for the separation of carbohydrates by a method called fluorophore-assisted carbohydrate electrophoresis (FACE). FACE requires that the analytes be labeled with a fluorophore; it is not capable of separating saccharides of similar size and charge and tends to be limited to reducing sugars.

Given the obvious need to develop a separation tool for saccharides of similar mass, we developed boron affinity saccharide electrophoresis (BASE).^{48a} Previous polymer-based sensors were used as design templates for the new technique. A range of acrylamide hydrogels were prepared that contained *o*-, *m*-, and *p*-boronic acids up to about 3% by weight. For practical applications, the meta derivative is preferred. FACE is not able to separate a series of 2-aminoacridone-labeled saccharides, while the BASE system allows for separation of the saccharides. Importantly, by use of the BASE system it was possible to separate previously inseparable saccharides.^{48b}

BASE was used to measure the modification of a protein by D-gluconolactone. The protein with a 25-residue N-terminal tag, (MSYHHHHHHDYDIPTTENLYFQGAM),²⁰⁸ has been shown to be especially prone to 6-phosphogluconoylation (6PGL) via mass spectrometric analysis. N-Terminal adducts were detected by mass spectrometry of the protein (an increase in mass of 258 Da, representing 6PGL, and/or an increase in mass of 178 Da, corresponding to the D-gluconolactone adduct after dephosphorylation).²⁰⁹ Therefore, we decided to use our BASE technique for protein that was incubated with gluconolactone (over various periods of time), and electrophoretic analysis was performed by standard polyacrylamide gel electrophoresis (PAGE) and protein BASE (Pro-BASE) or methacrylamido phenylboronate acrylamide gel electrophoresis (mP-AGE). The protein incubated with gluconolactone reveals a new band in mP-AGE that could not be resolved from the main band in the normal PAGE electrophoresis experiment.

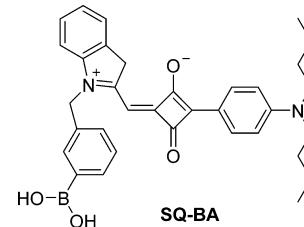
Electrophoresis combined with boronic acid derivatives seems promising for the analysis of monosaccharides²¹⁰ and glycoproteins in the detection of glycated hemoglobin;²¹¹ as a powerful proteomic tool, it provides the ability to identify potential biomarkers for age-related diseases.^{48b-d}

Capillary electrophoresis has also been used to probe the interactions between boronic acids and *cis*-diol-containing biomolecules. The interaction of 14 different boronic acids and 5 typical monosaccharides was comprehensively evaluated.²¹²

Coyer and co-workers²¹³ have linked boronic acid with a squarylium cyanine (long-wavelength) dye for on-capillary labeling of Gram-positive bacteria via boronic acid complexes with sugar chains on the bacteria surface. The system **109** used laser-induced fluorescence to measure Gram-positive bacteria. The method is very sensitive and compares favorably with traditional fluorescence stains. Subsequently, they used the long-wavelength squarylium cyanine as a fluorescence sensor for saccharides and glycoproteins in solution.²¹⁴

4.10. Electrochemical Systems for Sensing Applications

Electrochemical sensing systems based on boronic acid receptors are mainly based on direct effects of analyte binding in voltammetric experiments. An excellent review highlights the



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role of phenylboronic acids in electrochemical saccharide sensing.²¹⁵ Boronic acid derived electrochemical assays can be divided into two types: solution-phase and surface-immobilized processes.

4.10.1. Solution-Phase Processes. The heart of most current commercial D-glucose biosensors is the enzymatic decomposition of saccharides.²¹⁶ While boronic acid-based electroactive saccharide receptors for D-glucose could be developed, the main value of the synthetic boronic acid-based systems is that they could be used to develop sensors selective for a range of saccharides and are not limited to D-glucose sensing.

Soluble ferrocenylboronic acid redox probes have been extensively investigated; they were first synthesized by Nesmeyanov et al.²¹⁷ and have been used for direct electrochemical sensing of saccharides in aqueous media.²¹⁸ Reversible binding of saccharides was studied as a function of pH²¹⁹ and for various diols.²²⁰ A typical differential pulse voltammetry experiment for sorbitol binding is shown in Figure 14.

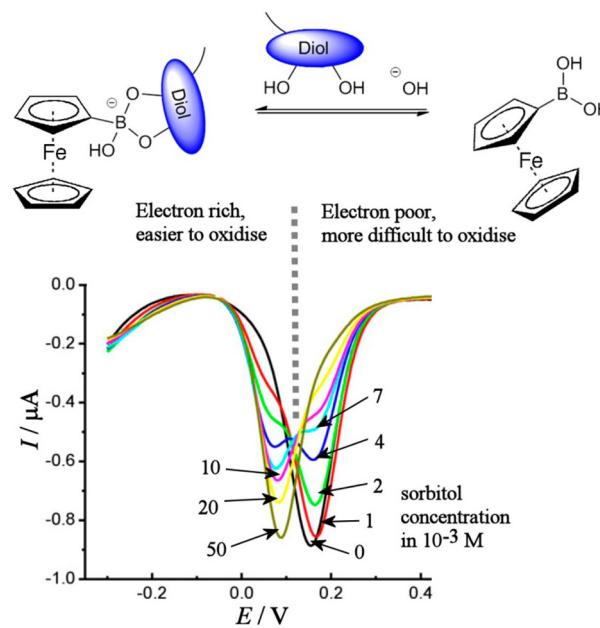


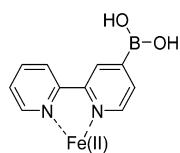
Figure 14. Binding of a diol to ferroceneboronic acid and a differential pulse voltammetry data set for sorbitol binding associated with a shift in reversible potential. Reproduced with permission from ref 220, copyright 2011 Elsevier, and ref 45i, copyright 2013 American Chemical Society.

Ori and Shinkai²¹⁸ have synthesized chiral ferroceneboronic acid derivatives and evaluated them for chiral electrochemical detection of monosaccharides. The best discrimination was observed for L-sorbitol and L-iditol at pH 7.0 in 0.1 mol·dm⁻³ phosphate buffer solution. Moore and Wayner²¹⁹ have used the redox switching of commercial ferroceneboronic acid on

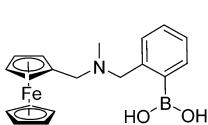
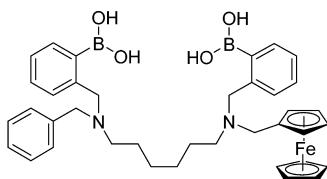
saccharide binding. These detailed investigations have indicated that the binding of saccharides with the ferrocenium form are about 2 orders of magnitude higher than the ferrocene form. The lower pK_a of the ferrocenium (5.8) compared to the ferrocene (10.8) boronic acid is believed to be the reason for the enhanced stability.

Electrochemical detection of lipopolysaccharides (LPS) has been achieved by Niwa and co-workers,²²¹ by use of ferrocenylboronic acid and an enzyme-modified electrode. The electrode prepared produced a rapid response to LPS, and the limit of detection (LOD) for LPS from *Escherichia coli* O127:B38 was 50 ng/mL.

Electrochemical sensing properties of boronic acid-substituted bipyridine iron(II) complex **110** have been evaluated by Fabre and co-workers.²²² Addition of 10 mM D-fructose to **110** resulted in the oxidation peak being shifted by 50 mV toward more positive values.

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Ferrocene monoboronic acid **111** and diboronic acid **112** have been used as electrochemical sensors for saccharides.²²³ The monoboronic acid system **111** was suggested as a potential electrochemical sensor for saccharides by Norrild and Sotofte.²²⁴ Electrochemical saccharide sensor **112** contains two boronic acid units (for saccharide selectivity), one ferrocene unit (for electrochemical readout), and a hexamethylene linker unit (for D-glucose selectivity). The diboronic acid electrochemical sensor **112** is selective for D-glucose (40 times) and D-galactose (17 times) over the comparable monoboronic acid sensor **111**.

**111****112**

Environmental analysis of diols and phenolics by ferrocenylboronic acids via electrochemical methods has been proposed.²²⁵ Several mono- and diferrocenyl complexes have also been fully characterized,²²⁶ and novel ferrocenylboronic acids have been proposed for fluoride,²²⁷ and glucose or saccharide detection.^{223,224} Chiral ferrocenylboronic acids have been reported for chiral determination by electrochemical methods.²²⁸ Boronic acid groups attached to other redox probes such as iron–bipyridyl complexes,²²² phenazines,²²⁹ and tetrathiafulvalenes²³⁰ have also been prepared. Interestingly, in some cases the analyte itself has been used as the redox probe, for example, catechols when bound to phenylboronic acid.²³¹

4.10.2. Surface-Immobilized Processes. Electrochemical processes are fundamentally heterogeneous in nature; therefore, sensing systems with enhanced sensitivity and increased selectivity occur directly at the electrode solution interface.

A large body of research has been devoted to the study of conducting polymers, in films of polypyrrole^{222,232} and polyaniline,²³³ with covalently bound phenylboronic acid. These polymer-sensing films are rapidly prepared by electropolymeriza-

tion,²³⁴ and the nature of the polymer matrix has been employed to enhance selectivity.²³⁵

Interaction of ferrocenylboronic acid with surface-immobilized hemoglobin protein has been demonstrated as an excellent method to determine protein glycation levels in blood with an amperometric biosensor.²³⁶ Nanostructuring has produced improved sensitivity, such as surfactant templating to give poly(aniline) boronic acid nanofibers²³⁷ or by controlled copolymerization.²³⁸ Multiwalled carbon nanotubes functionalized with boronic acids²³⁹ or formed into nanostructured composites²⁴⁰ have been demonstrated to exhibit high sensitivity toward o-quinols, including dopamine. Carbon nanotubes functionalized with 3-aminophenylboronic acid have been shown to capture leukemia cells and can serve as a reusable cytosensor.²⁴¹

Monolayer films of boronic acids at electrode surfaces have been prepared via diazonium grafting to glassy carbon²⁴² and by use of self-assembled monolayers (SAMs) on gold:²⁴³ for example, self-assembly of amphiphilic N-hexadecylpyridinium-4-boronic acid cations onto a monolayer graphite electrode.^{243d} The observed binding constants for a series of o-quinols indicate selectivity for the hydrophobic dye alizarin red S.

Also, self-assembled monolayers constructed from 3-amino-phenylboronic acid on gold have been used to develop immunosensors;^{241,244} here the boronic acid plays a structural role. Self-assembled boronic acid dendrimers with nanocellulose whiskers have also been prepared and evaluated.²⁴⁵

A biphasic redox system consisting of microdroplet deposits of water-insoluble organic liquids at electrode surfaces has been used together with dissolved boronic acids. In this liquid–liquid system, hydrophobic (naphthalene or anthracenyl) boronic acid derivatives are solubilized in microdroplets of 4-(3-phenylpropyl)pyridine. The tetraphenylporphyrinato-manganese(II/III) redox system then allows anions to be actively transferred from aqueous to organic phase. By use of these systems, the transfer of carbonate and bicarbonate,²⁴⁶ as well as the transfer of α -hydroxycarboxylates, has been reported.²⁴⁷ Selectivity of the boronic acid is combined with the selectivity effect introduced by the Gibbs energy for anion transfer from aqueous into organic phase. These biphasic redox systems with boronic acids could potentially be developed into hydrophobic membranes for analytical and separation purposes.

Electrochemical sensing methods are highly advantageous for the transduction of surface-confined boronic acid–diol binding events. For instance, the electrochemical impedance of a boronic acid-derivatized electrode surface responds to the presence of glucose.²⁴⁸

5. CONCLUSIONS

The number of adults living with diabetes continues to increase and is a major worldwide health problem. The majority of diabetics have type 2 disease, which is tied to a poor diet, lack of exercise, and obesity; it is predicted that one in 10 adults will suffer from diabetes by 2030. This poses a huge challenge for worldwide healthcare systems and will result in an increasing financial burden on the working-age population (which is on the decline). This may ultimately mean that maintenance of cradle-to-grave government-funded healthcare may not be possible in the long term. Therefore, the development of any new, or improved diagnostic technologies, including supramolecular chemistry, that can be used to improve the long-term prognosis of diabetic care and reduce the overall cost of treatment, will have enormous economic and healthcare impacts. The importance of carrying out

Table 1. Boronic Acid-Based Fluorescent Sensors for Monosaccharides^a

saccharide	binding constants K (M^{-1})							
	monoboronic acids		diboronic acids			cationic boronic acids		aggregate
	36 ^{66,71b}	37 ^{67a}	46 ^{85a,b}	38 ⁷¹	53 ^{86c}	40 ⁷⁴	97 and 98 ¹⁵¹	89 and 90 ^{88b}
Fructose	784 (3.2)	480 (0.7)	784 (3.2)	320 (7.5)	34 (3)		5.0×10^4 (51)	1100 (2.6)
Glucose	44 (4.5)	38 (0.5)	962 (2.8)	4000 (7.5)	1472 (8)	4.0×10^4 (0.5)	33 (31)	1900 (2.0)
Galactose	51 (4.2)		657 (3.1)	160 (6.0)	30 (3)	100 (0.7)	167 (68)	180 (2.1)
Mannose	36 (3.7)		74 (2.8)			62.5 (0.8)		24 ± 3 (1.73)
$K_{\text{Glu}}/K_{\text{Fru}}$	0.06	0.08	1.2	13	43		0.0007	1.7
								77 ± 9 (1.93)
$K_{\text{Glu}}/K_{\text{Fru}}$	0.06	0.08	1.2	13	43		0.0007	1.7
								3.9

^aNumbers in parentheses denotes maximum fluorescence change. ^b K (M^{-2}) was obtained by assuming boronic acid:saccharide = 1:2. ^c K is square-rooted for direct comparison.

effective monitoring of glucose levels in blood of diabetic patients cannot be overemphasized, since it has been estimated that an additional five years of life, eight years of sight, and six years free from kidney disease and amputations can be gained by a diabetic patient who maintains tight glucose control regimes.

In a 2013 review, Wolfbeis²⁴⁹ stated that "It is a matter of fact that the overoptimistic claims on boronic acid based sensor chemistry for glucose have come to no avail so far, but remained only 'on paper'". However, given the successful human trials conducted by Glysure Ltd. (www.glysure.com) with **49** (cf. **46**), that statement is no longer correct. Also, while boronic acid-based systems have taken a considerable amount of time to be developed to a point where they can be used in devices, they have from their inception been tasked with the very difficult problem of continuous glucose monitoring (CGM). Given the success in continuous glucose monitoring achieved with sensor **49**, a comparison of the model system sensor **46** with several other fluorescence sensor designs discussed throughout this review is shown in Table 1. It is anticipated that this table will be a helpful guide for researchers working on the development of novel D-glucose-selective fluorescent boronic acid-based sensors. While the data in Table 1 are for fluorescence-based sensors, the binding constants are valid for any sensing mechanism. Therefore, the data given in Table 1 for **46** can be used as a benchmark to which any new system can be compared in order to decide if the system is suitable for further development into a practical D-glucose sensor system.

From Table 1, sensor **46** is the most similar to the Glysure Ltd. system (**49**) and so can act as a base system by which to compare other systems. Clearly the monoboronic acid systems **36** and **37** are not suitable, since the binding constant for glucose is small and the selectivity is for fructose. The same is true for **89** and **90**; however, this system does produce large fluorescence enhancements (modulation) upon interaction with saccharides. This is important because, for a practically useful sensor, not only selectivity and sensitivity but also a large modulation of fluorescence with changes in the concentration of glucose are required. Interestingly, the remaining systems in Table 1 all have larger binding constants and greater selectivity for glucose over fructose than sensor **46**. This clearly illustrates that while selectivity and strong binding constants are important, other factors come into play and cannot be ignored when preparing a practically useful sensor. These factors include but are not limited to the wavelength of fluorescence and sensitivity to other environmental factors such as pH.

The wider implications of success of glucose sensing in supramolecular chemistry will be of immeasurable benefit to society, since the science lies at the leading edge of discoveries in

diagnostics, medicine, and healthcare and will provide core sensing technologies for future applications, perhaps for the development of an artificial pancreas for real-time closed-loop control of blood glucose levels.

AUTHOR INFORMATION

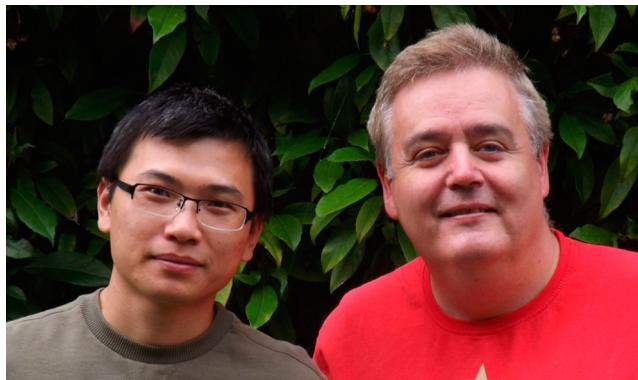
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Notes

The authors declare no competing financial interest.

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Xiaolong Sun received his M.Sc. in 2012 from East China University of Science and Technology under the supervision of Professor Xuhong Qian. In 2012, he joined the chemosensor group led by Professor Tony D. James in the Department of Chemistry, University of Bath, as a Ph.D. student. His research interests include organic synthesis to prepare small molecules that can be utilized to understand and exploit biological systems, mainly in the fluorescent detection of reactive oxygen and nitrogen species and carbohydrates such as monosaccharides.

Tony D. James is a professor at the University of Bath. He obtained his B.Sc. from the University of East Anglia in 1986 and Ph.D. in 1991 from the University of Victoria and worked as a postdoctoral research fellow in Japan from 1991 to 1995 with Seiji Shinkai. From 1995 to 2000 he was a Royal Society Research Fellow at the School of Chemistry at the University of Birmingham, moving to the Department of Chemistry at the University of Bath in September 2000. He has been a visiting professor at Tsukuba, Osaka, and Kyushu Universities, an AMADEus invited professor at the University of Bordeaux, and is a guest professor at East China University of Science and Technology, Xiamen University, Shandong Normal University, and Nanjing University. He is a Hai-Tian (Sea-Sky) Scholar at Dalian University of Technology and in 2013 he was awarded the Daiwa Adrian Prize. His research interests include many aspects of supramolecular chemistry, including molecular recognition,

molecular self-assembly, and sensor design. Within the area of molecular recognition, his research has a particular focus on boronic acid-based receptors for the fluorescence sensing of saccharides.

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