Boronic acid building blocks: tools for sensing and separation

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In this feature article the use of boronic acids to monitor, identify and isolate analytes within physiological, environmental and industrial scenarios is discussed. Boronic acids recognise diol motifs through boronic ester formation and interact with anions generating boronates, as such they have been exploited in sensing and separation protocols for diol appended molecules such as saccharides and anions alike. Therefore robust molecular sensors with the capacity to detect chosen molecules selectively and signal their presence continues to attract substantial attention, and boronic acids have been exploited with some success to monitor the presence of various analytes. Reversible boronic acid-diol interactions have also been exploited in boron affinity chromatography realising new separation domains through the same binding events. Boronic acid diol and anion interactions pertaining to sensing and separation are surveyed.

1. Introduction

In 1860 Frankland published the first synthesis of an organo-boron compound, ethylboronic acid.[1] Twenty years later dichlorophenyl borane was prepared by Michaelis and Becker, from which phenylboronic acid was prepared.[2] Subsequently trialkyl borates were used to prepare boronic acids from Grignard reagents in the *classical* synthesis we know today.[3] The exploitation of boronic acids' interactions has seen an explosion of applied boronic acid based systems in sensing,[4] self-assembly[5] and separation science,[6] some recent developments pertaining to sensing and separation are discussed herein.

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1.1 Scope of article

This feature article concentrates on the recent developments in the boronic acid arena pertaining to sensing and separation. This article represents one of a two part contribution, self assembly facilitated by boronic acids is discussed in the partner manuscript.[7] Presented discussions highlight, but are not limited to, research of the co-authors, and whilst not exhaustive attention is paid to recent work in the area by others as well as the seminal and historically key publications which set the scene for subsequent discussion.

2. Boron's interactions

2.1 Boron–diol interaction

Since boric acid is significant in determining saccharide configurations,[8] it is a little surprising that the same properties were not reported with boronic acids until 1954.[9] An investigation into aromatic boronic acids by Kuivila and

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co-workers revealed a new compound formed on addition of phenylboronic acid to a saturated solution of mannitol, and correctly postulated the formation of a cyclic boronic ester analogous to the one known to form between boric acid and polyhydroxyls. An intriguing hypothesis has recently emerged relating theories pertaining to the origins of life on this planet with an implicated role of boron and its interactions with sugars.[10]

Reports detailing the synthesis and properties of boronic acids appeared after the initial reports,[11] with quantitative studies into the interactions between boronic acids and polyols first being reported in 1959.[12] The tetrahedral, rather than trigonal structure of phenylboronic acid conjugate base was proposed by Lorand and Edwards. The dissociation of a proton from phenylboronic acid occurs from the interaction of the boron atom with a molecule of water. As the phenylboronic acid and water react a hydrated proton is liberated, thereby defining the acidity constant K_a.[13–15] The reported pK_as of phenylboronic acid ranged between ~8.7 and 8.9,[16] and in-depth potentiometric titration studies refined this value to 8.70 in water at 25 °C.[17]

Diols react with boronic acids to form boronic esters in aqueous media,[12,18] and it was believed that the kinetics of this interconversion were fastest in aqueous basic media where the boron is present in its tetrahedral anionic form.[19] It should also be noted that Ishihara reported reaction rate constants of boronate ion with aliphatic diols to be much smaller than those with boronic acid.[20] Whilst six-membered cyclic boronic esters can be formed with 1,3-diols, the stability of these diesters is lower than their five-membered analogues.[19,21]

2.2 Boron-nitrogen interaction

Dative nitrogen boron interactions were first reported when a complex formed between ammonia and trimethylborane, was discovered in 1862.[22] Early examples of intramolecular N → B bonds reported in 1968 by Dunn *et al.*[23] are the imines formed between *ortho* formylphenylboronic acid and amines.

The recognition of saccharides through boronic acid complex (or boronic ester) formation often relies on an ancillary interaction between Lewis acidic boronic acid and a proximal tertiary amine (Lewis basic). Confirmation of the true nature of the nitrogen–boron (N–B) interaction has been the subject of debated, but it is clear that an interaction of some kind exists which offers two advantages.[17,24] It was proposed by Wulff that a reduction in boronic acid pK_a results from a boron–nitrogen interaction,[25] facilitating binding at neutral pH, thus extending the applications scope beyond the high pH arena. Secondly narrowing of the O–B–O bond angle upon complex formation with a diol manifests an increase in boron's Lewis acidity. The acidity increase of the Lewis acidic boron enhances the N···B interaction which, in certain systems, can modulate the fluorescence of nearby fluorophores, which is extremely useful in the design and application of chemosensors.

In a detailed study of 144 compounds with N–B coordinative bonds steric interactions along with ring strain (in the case of cyclic diesters) were concluded to weaken and elongate the N–B bond, as well as a reduction in tetrahedral geometry at boron.[26] An *N*-methyl-*o*-(phenylboronic acid)-*N*-benzylamine system has been investigated separately by leaders in the field.[17,24*d*,25,27] From these reports it can be seen that the upper and lower limits of the N–B interaction lies between ~15 and ~25 kJ mol^{-1} in the *N*-methyl-*o*-(phenylboronic acid)-*N*-benzylamine system.[17] A computational study estimated the N–B interaction to be 13 kJ mol^{-1} or less in the absence of solvent.[28] thus a N–B interaction may be considered to be of similar strength to a hydrogen bond. Computational and potentiometric titration data also point to the fact that intramolecular seven-membered rings should not be ignored.[16*b*,17,29] Infrared spectroscopy suggests the N–B interaction in a related system draws a similar with a "tentative conclusion,"[30] from an experimental rationale which compares two emergent peaks in IR spectra to peaks of model systems.

Anslyn carried out in depth structural investigations of N–B interactions on *o*-(*N*,*N*-dialkyl aminomethyl)

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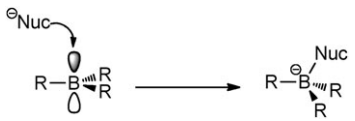
arylboronates.[24a] ^{11}B-NMR spectroscopy (and XRD data) revealed that a dative N–B bond is usually present in aprotic media. Although, protic solvents may insert into the N–B bond generating a hydrogen-bonded zwitterionic species. Wang and co-workers suggested solvolysis rather than N–B bond formation could happen upon sugar binding.[28] Thanks to these investigations,[17,24a,d,28,31] the N–B interaction can be defined in many cases as a hydrogen bonding interaction regimented by a solvent molecule.

2.3. Boron-anion interaction

In 1967 when Shriver and Biallas,[32] identified a complex formed between a bidentate Lewis acid and a methoxide anion, the converse of the circumstances of a metal ion accepting electron density from a difunctional base.

Trisubstituted boron atoms have an sp^2 trigonal planar geometry with an empty p orbital. Nucleophiles interact with or donate electron density to the vacant site, resulting in a change of geometry and, importantly, hybridisation (Scheme 1). The tetrahedral geometry of the phenylboronate anion was established by Lorand and Edwards in 1959 (see section 2.1).[12]



Scheme 1 Diagram showing the change in geometry undergone at the boron centre when the vacant p orbital is filled by an attacking nucleophile.

Not only does the somewhat weak Lewis acidity of boron facilitate an array of synthetic chemistry but also allows the boron centre to act as a receptor for anions, such as hydroxide, cyanide and fluoride. The Brønsted acidity of boron becomes apparent when considering covalent interactions. Since the pK_a of phenylboronic acid is 8.70 in water at 25 °C,[33] boronic acids reversibly and rapidly react with diols to give cyclic boronate esters under non-aqueous or high pH aqueous conditions.[34] Boronic acids also show significant affinity for other nucleophiles such as α-hydroxy-carboxylic acids[35] and dicarboxylic acids.[34a,36] Boron, due to its Lewis acidic nature, forms coordinate bonds with a range of hetero-atoms such as sulfur,[37] oxygen, nitrogen[38] and phosphorus,[39] with widespread use in synthesis.[40]

3. Boronic acids as sensors

3.1 Anion sensors

Anions are involved in fundamental processes in all living things. Recognition, transport and concentration control of anions such as chloride, phosphate and sulfate is carried out by biological systems on a continual cycle. While fluoride, nitrate and pertechnetate anions are potentially dangerous contaminants that can gain access to our water systems by various means.

Chemosensors capable of determining the concentration of a target anion in any given medium are the are a valuable tool in the detection and determination of these important analytes.

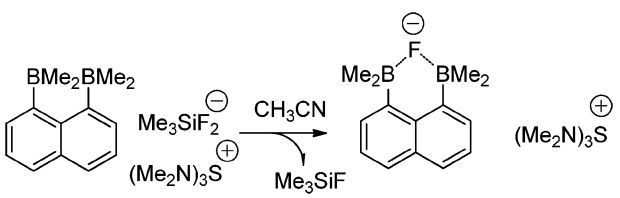
3.1.1 Boron anion interactions. The first detailed investigation of Lewis acidic boron binding to fluoride ion was published in 1985 by Katz (Scheme 2). The fluoride adduct was characterised successfully by ^{19}F–^{1}H and ^{19}F–^{13}C coupling in its NMR spectra. For the adduct on binding fluoride, the B–B distance was found to be significantly shorter and an sp^3 character at the boron centres was indicated by ^{11}B NMR spectroscopy.[41] Katz also prepared an analogous system where one boron is replaced by a trimethyl silyl group and using X-ray analysis found that the fluoride bridges the boron and silicon.[42]

In 1991 Reetz *et al.* observed that a crown ether, the archetypal cation binding skeleton, appended with a Lewis acidic boron centre (**1**), served to solubilise a stoichiometric amount of a suspension of KF in dichloromethane.[43] The proposed dual host–guest system (Scheme 3) was examined by ^{11}B and ^{13}C NMR spectroscopy. The ether carbon atoms of the crown ether display a low-field shift due to cation binding. The complex also displays an upfield shift in the ^{11}B NMR spectroscopic signals from 30 ppm characteristic of sp^2 boron to 10 ppm, indicating a pseudo tetrahedral sp^3 environment at boron. KCl and KBr did not bind monotopically or heterotopically even after 2 weeks. The system was also very selective, since, in the presence of the potassium salts of fluoride, chloride, bromide and iodide, only the fluoride adduct was observed.

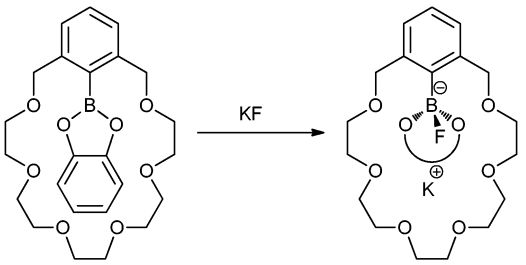
3.1.2 Boron Lewis acids as fluoride sensors. In 1995 Shinkai showed that the interaction between Lewis acidic boron and strongly basic fluoride could be exploited to create a means of determining the concentration of fluoride anions in aqueous solution, even in the presence of other anions including halides.[44] This landmark discovery was the first example of transforming a receptor unit for fluoride anion into a chemosensor. The pK_a values of ferrocene and ferrocenium are 10.8 and 5.8 respectively, the lower pK_a of the ferrocenium cation of **2** (Fig. 1) increases the affinity of the boron for fluoride whilst simultaneously providing the redox active centre.[45]

Increasing the concentration of fluoride ions results in a decreasing polarographic half wave potential which is linear over a 200 mM range. In 9 : 1 H_2O : MeOH, the selectivity for fluoride over chloride was 500-fold and was selectivity over sulfate anions was 50-fold. Hydroxide interacts with the boron center in a similar fashion to fluoride but this only occurs at high pH *i.e.* when the concentration of hydroxide is high.

A related system **3** (Fig. 1) containing two boronic esters has been developed by Aldridge *et al.*, who observed that the bis fluoride adduct undergoes a colour change from orange to green as the ferrocene is aerobically oxidised to ferrocenium.[46] The same group has also developed a boronic acid dimethyl amine conjugate **4** (Fig. 1) to act as a ditopic receptor for HF.[47]



Scheme 2 The first Lewis acid based bidentate fluoride receptor.



Scheme 3 A ditopic receptor for potassium fluoride based on a crown ether.

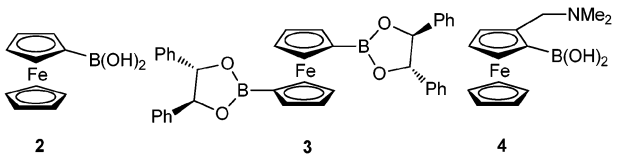


Fig. 1 Ferrocene based receptors for fluoride **2**, **3** and ditopic receptor for HF **4**.

Shinkai[48] futher elaborated the ferrocenyl system and created a colourimetric sensor capable of visually detecting fluoride. Taking advantage of a redox reaction between the dye molecule Methylene Blue and ferrocenyl boronic acid, it was possible to visually determine fluoride concentrations. Decolourisation of the dye occurs over a 4×10^{-3} mM to 3×10^{-2} mM range (monitored by UV-Vis spectroscopy by a decreasing absorbance at 665 nm).

In 1998 the first fluorescent sensors with a selectivity for fluoride were reported.[49] Fluorescence quenching of a series of simple aromatic boronic acids (Fig. 2) was observed in buffered aqueous methanol solution at pH 5.5 upon addition of KF. Tetrahedral boronate anions had already been shown to quench the fluorescence of directly attached fluorophores and this same ICT mechanism was shown to proceed upon fluoride binding.[50] The ^{11}B NMR spectroscopic observations of **5** and **6**, (Fig. 2) displayed shifts consistent with a change from an sp^2 to sp^3 boron centre as the concentration of fluoride was increased from 1 to 5 equivalents.

Compounds **5** and **6** (Fig. 2) allow fluoride concentrations to be determined over a 50–70 mM range. With **7** (Fig. 2) a tertiary amine component was introduced to provide an additional hydrogen bonding site. The amine proton of **7** (Fig. 2) has a pK_a of 5.5 so under the measurement conditions the nitrogen is partially protonated, allowing a hydrogen bonding interaction with the fluoride when bound to the boron **8** (Fig. 2). The two binding sites of **8** (Fig. 2) enhanced the binding permitting determination of fluoride at lower

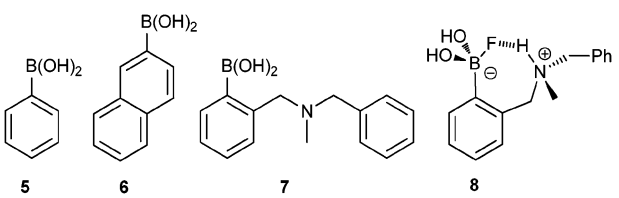


Fig. 2 Early examples of fluorescent sensors for fluoride.

concentrations (5–30 mM). This family of compounds serves to introduce the concept of *tuneability*. Since, different environments require monitoring across varying concentration ranges, only simple modifications need to be made in order to enhance binding without changing the mode of action. A fluorescence sensor closely related to **7** (Fig. 2) was prepared by Yoon *et al.* (Fig. 3) where the fluorophore was a fluorescein motif; this system produced large fluorescence and a visible response to added fluoride.[51]

In order to enhance fluoride binding the use of a rigid framework as a scaffold for boronic acid based anion sensors was investigated. It was found that the bis(bora)calix[4]arene **9** (Fig. 4) acts as a sensor for tetra-*n*-butylammonium fluoride (Bu_4NF) in chloroform.[52] Subsequently, in order to probe the factors affecting fluoride binding related boronates **12** and **13** were prepared (Fig. 4).[53]

On addition of an excess of Bu_4NF to solutions of **12** or **13** in chloroform dramatic colour changes from colorless to yellow (**12**) or purple (**13**) were observed. In both cases, ^{1}H NMR spectroscopic analysis clearly indicated Bu_4NF-mediated cleavage of the boron–aryloxide bond, an observation in agreement with a report of Bresner *et al.*[54] These observations led to an investigation of the addition of fluoride to the parent phenols **10** and **11** (Fig. 4), which also exhibited similar colour changes to those observed with **12** and **13** (Fig. 4). In light of the similar behaviour of both phenols and arylboronates in the presence of fluoride, a feasible mechanism for this colourimetric response involves a common phenolate anion intermediate obtained *via* either fluoride-mediated deboronation or deprotonation, followed by ambient oxidation to a coloured radical, that is stabilised by the 2,6-dialkyl substitution. The importance of radical formation in the generation of coloured species was established using electrochemical techniques.[53]

While fluoride caused deboronation of compound **13** (Fig. 4) and dramatic colour changes, Bu_4NCl and Bu_4NBr produced no colour change. Bu_4NCl caused fluorescence quenching of compound **13** but did not quench **12**, or alcohols **10** and **11** (Fig. 4). Bu_4NBr did not cause a significant change in the fluorescence spectra of compounds **10–13** (Fig. 4). The fluorescence quenching by chloride has been attributed to bidentate binding through two BOH hydrogen bonds, with the conformational change in the fluorophore causing the fluorescence quenching.

In 2001, Yamaguchi *et al.* reported a range of boron-containing species **14a–d** (Fig. 5) that showed a visible colour

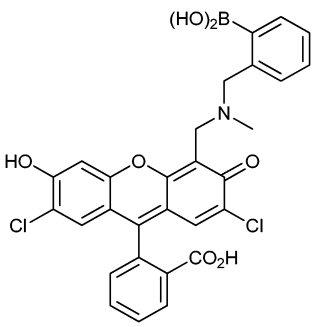


Fig. 3 Fluorescein based fluorescent sensor for fluoride.

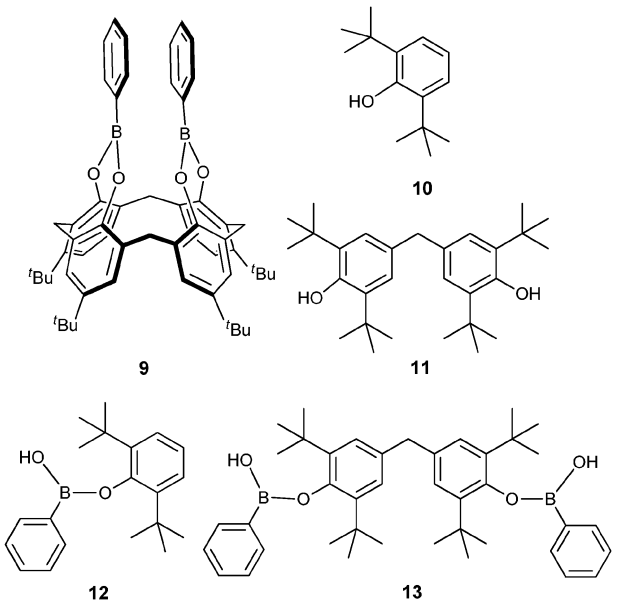


Fig. 4 Structure of calixarene a (rear *tertiary* butyl groups removed for clarity) and related compounds b, c, d, and e.

change upon fluoride binding in THF.[55] The highly conjugated system is disrupted by the boron–fluoride interaction and hybridisation change of the boron from sp^2 to sp^3.

Shinkai prepared a colourimetric and ratiomeric fluorescence chemosensor **15a** (Fig. 6) that displayed three emission responses to fluoride ions at 356, 670 and 692 nm.[56] The system comprised of a porphyrin and a triarylborane centre connected *via* a conjugated linker (Fig. 6). Changing the conjugation of a laterally expanded porphyrin results in a significant hypsochromic shift of the Soret band and a bathochromic shift of the Q band.[57]

Fluoride coordination to the boron centre generates an anionic sp^3 boron disrupting the linker conjugation and causing a change in the energy pathway. Evidence for the latter finding was provided by measuring the fluorescence decay of the emission band. The fluorescence lifetime at 515 nm of free **15a** (Fig. 6) corresponds to less than 100 ps, this lengthened to 0.53 ns for the fluoride bound species. Compound **15b** (Fig. 6) which does not contain a porphyrin linked component, has a longer lifetime than that of **15a** (Fig. 6), at 4.52 ns. The shorter lifetime of **15a** (Fig. 6) can be rationalised by Dexter-type energy transfer from the triaryl

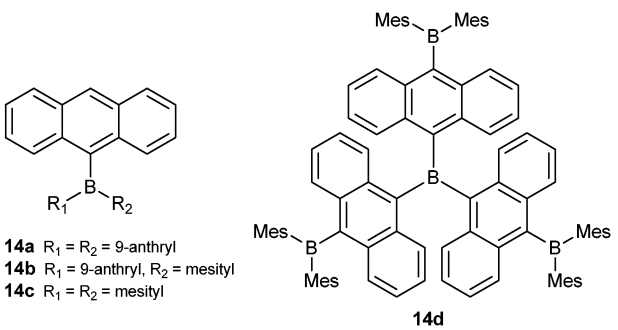


Fig. 5 Triaryl borane species can act as colourimetric fluoride sensors.

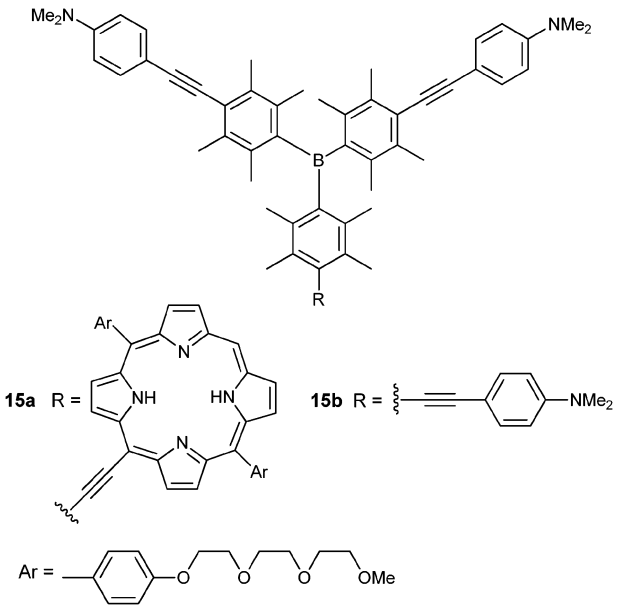


Fig. 6 Ratiomeric sensors **15a** and **15b**.

borane to the porphyrin ring system. While across the host–guest complex **15a**.F^- (Fig. 6) only limited energy transfer can occur.

For the majority of reported fluorescent sensors of fluoride, the binding of the anion causes a quenching of the fluorescence emission.[58] Only a few sensors in which the binding of a fluoride ion causes an increase in the fluorescence have been reported.[59] However, in most practical applications, changes in fluorescence intensity (fluorescence quenching or enhancement) can also be caused by many other poorly quantified or variable factors such as photobleaching, sensor concentration, the environment around the sensor molecule (polarity, temperature and so forth) and the stability of the sensory system under illumination. To increase the selectivity and sensitivity, ratiometric measurements are utilised, which involve the observation of changes in the ratio of the intensities of the absorption or the emission at two wavelengths. Ratiometric fluorescent probes have the important feature in that they permit signal rationing and thus increase the dynamic range and provide built-in correction for environmental effects.[56,60]

Gabbaï is at the forefront of the challenging prospect of anion recognition in aqueous media. One of his first forays into this area came in 2004 when a neutral bidentate diborane species was used to bind fluoride, creating a colourimetric sensor (Fig. 7) that is not affected by the presence of water. A rigid 1,8-naphthalene backbone with two proximal Lewis acidic sites promoting fluoride anion chelation is the key to the

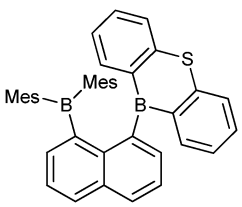


Fig. 7 Gabbaï's first bidentate system for fluoride.

utility of these systems. The association constant of the bis boron species (Fig. 7) with fluoride, was 5×10^9 M^{-1} in THF, which was higher than that observed for any previously documented monofunctional borane receptor. Addition of $B(C_6H_5)_3$ permits conversion back to the unbound sensor, confirming reversible binding.[61] The visual cue to the binding event is the dissipation of the vivid yellow colour, recordable as a decreasing absorption at 340–390 nm by UV spectroscopy.

The bridging fluoride species $[\mu_2\text{-F}]^-$ was successfully isolated and characterised, bidentate binding was confirmed by shifts in the ^{11}B NMR spectrum, and the expected −188 ppm ^{19}F NMR spectroscopic signal. X-Ray crystal structure analysis confirmed the two B-F bonds and pyramidal boron centres.

Liu has reported a highly sensitive and selective sensor for fluoride based on an organic borane (Fig. 8).[62] A colour change of bright green to colourless accompanies fluorescence quenching on fluoride binding. The dimesityl tri-coordinated boron species showed both two-photon excited fluorescence (TPEF) and single-photon excited fluorescence (SPEF) activity on binding with fluoride. The trigonal planar boron is shown to be well shielded by the dimesityl groups, possibly increasing the selectivity towards fluoride ion due to its small size. TPEF chemosensors have been used in conjunction with laser scanning microscopy in the imaging of ions in cellular processes with greater 3D spatial selectivity than SPEF techniques.[63]

Most monodentate boron based chemosensors show a fluorescence quenching on binding, termed 'switch-off' sensors. However, practical applications would benefit from a 'switch-on' type response, particularly if accompanied by a visible colour change. The synthesis of two fluorescent sensors **16a** and **16b** (Fig. 9) were also reported. With **16a** it was observed that selective fluorescent enhancement for fluoride over other halides for in dichloromethane occured.[64]

Molecular modelling predicts that the two biphenyl 'arms' of compound **16a** sit orthogonally to the naphthalene ring, implying charge transfer through space from the 'donor' nitrogen to the dimesitylboron 'acceptor'. In solution, compound **16b** emits in the green region, upon binding to fluoride charge transfer is interrupted and a change to a characteristic blue colour is observed. Compound **16b** displays a typical fluorescence quenching behaviour, indicating the amine functionality and its interaction with the neighbouring boron centre was critical for the desired enhancement effect.

In perhaps the most compelling example of a fluoride sensor system to date, Gabbaï *et al.* synthesised an impressively simple series of sensors (**17**) that incorporated a charged phosphonium unit (Fig. 10) and a triarylborane. Binding

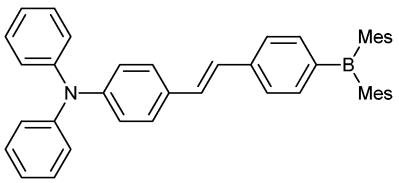


Fig. 8 Fluoride sensor incorporating bulky mesityl substituents intended to increase fluoride selectivity.

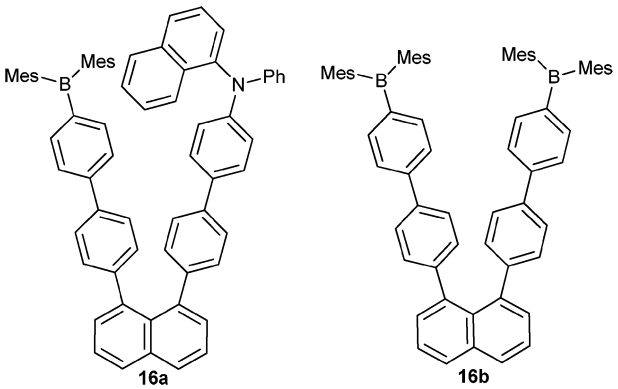


Fig. 9 Two related molecules that serve as examples of a "switch on" fluorescence sensor **a** and a "switch off" fluorescence sensor **b** for fluoride.

constants were calculated at optimum pH (pH 4.6 to 4.9) in 9 : 1 H_2O–MeOH giving a maximum value of 10.5×10^3 M^{-1} for species **17d**.[65] The increased Lewis acidity required to overcome the large hydration enthalpy (504 kJ mol^{-1}) of the fluoride anion in water has been proposed by the authors to be provided by the Coulombic effect of the cationic substituent.[66]

Across the series **17a–d** it was found that as the hydrophobic character increases, there is a corresponding increase in the Lewis acidity at the boron centre. To highlight the success of this body of work, compound **17d** is capable of binding fluoride in water at pH 4.9,[65] at concentration levels below the US Environmental Protection Agency's recommended maximum level for drinking water of 4 ppm.

3.1.3 Mixed or assisted Lewis acid systems. Sensors containing boronic acids (or derivatives thereof) as binding sites have not been limited to bearing this type of binding moiety exclusively. Several examples of additional functionality next to boron to either complement the binding or introduce additional signalling units have been reported.

Bidentate frameworks for anion sensing have been developed by Gabbaï and co-workers where they explored mixed Lewis acid centres and charged receptors for fluoride. They aimed to develop systems capable of working in aqueous media. In 2005, Gabbaï revealed a highly selective phosphorescent sensor Scheme 4 for fluoride similar to the sensor from Scheme 2 however an organomercury fragment was included.[67] Again, a $\mu_2\text{-F}^-$ bridged species (Scheme 4) was isolated and characterised fully by X-ray analysis. The distinct green solid state phosphorescence changed to red upon binding. With a binding constant higher than that measurable by direct titration in THF ($K_a > 10^8$ M^{-1}) this system also showed binding in aqueous media, albeit reduced, ($K_a = 3.3 \times 10^5$ M^{-1}). Even the highly competitive acetate anion did not bind, possibly due to the fact that the acetate does not have a μ_2-bridging binding mode.

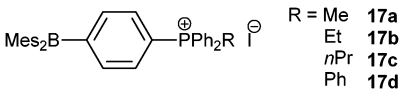
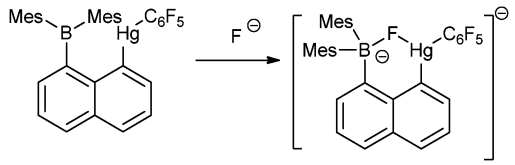


Fig. 10 A cationic borane capable of sensing fluoride in 90% aqueous media.

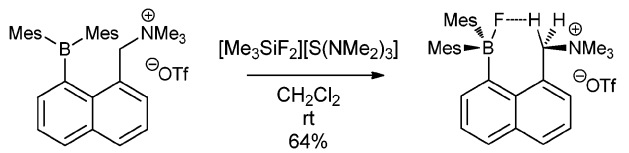


Scheme 4 Fluoride capture by a mixed Lewis acid system.

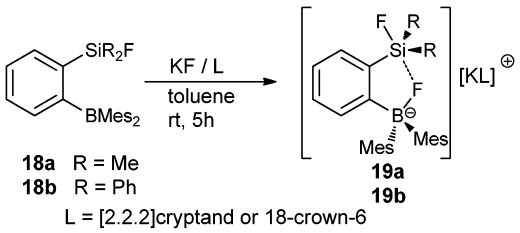
Phosphorescence is comparatively rarely utilised as an optical probe for anion sensors in comparison to fluorescence. However, in this example, the authors took advantage of mercury's ability to induce phosphorescence of hydrocarbon chromophores *via* spin–orbit coupling at room temperature. In a related report the synthesis of two very cationic bidentate Lewis acids were revealed, these were prepared in order to assess whether Coulombic attractions improved the binding constant. Stronger binding was observed with in the cationic version partially in aqueous-THF/H_2O (9 : 1).[68] However, this system suffered from a response to acetate ions, therefore a degree of selectivity was lost. Continuing their exploration into Coulombic factors, the same group showed cationic borane (Scheme 5) was capable of capturing fluoride ions across a phase barrier, extracting fluoride from water in a chloroform/water biphasic system.[69] X-Ray diffraction studies confirmed the presence of a C–H···F–B hydrogen bond arrangement contributing to the binding motif. A striking piece of evidence for this behaviour persisting in solution came from ^{1}H and ^{19}F NMR spectroscopy, paying particular attention to the emergence of diastereotopic methylene protons. One of the resonances showed coupling to the fluorine nucleus ($^{1}J_{H-F}$ = 9.2 Hz) and was resolved as a doublet of doublets ($^{2}J_{H-H}$ = 12.9 Hz).

The mixed silicon–boron Lewis acid systems **18a** and **18b** (Scheme 6), constructed around an *o*-phenylene backbone,[70] exhibited stronger binding than the comparative monodentate boron analogues. Fluoride-bound species **19a** and **19b** have been studied by X-ray crystallography and through Si–F coupling in ^{19}F NMR spectroscopy, which provided evidence of that silicon adopts a pseudo-pentacoordinate geometry and ^{11}B NMR spectroscopy indicated a strong tetrahedral character.

A ditopic receptor reminiscent of Reetz's crown ether boronic acid system (Scheme 3) has been developed (**20a** and **20b**, Fig. 11). Compounds **20a** and **20b** function as AND logic gates *via* their selectivity for potassium fluoride.[71] The sp^2 hybridised boronic acid, which is a hard Lewis acid, interacts strongly with a fluoride anion, which is a hard Lewis base, and becomes sp^3 hybridised. The potassium cation is held partly by the crown ether and partly by the electrostatic interaction with the fluoride anion. This co-operative complexation allows the cationic and anionic guests to be bound to the host as an ion pair, whilst allowing the host to discriminate between



Scheme 5 Combination of Lewis acidic boron interaction with a neighbouring hydrogen bonding interaction to selectively extract fluoride across a biphasic barrier.



Scheme 6 A mixed silicon–boron bidentate receptor for fluoride.

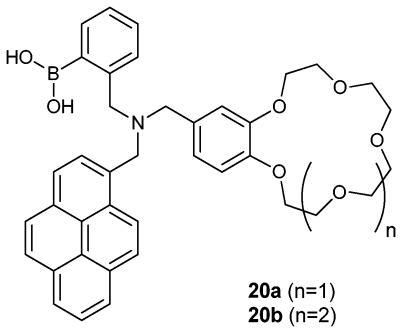


Fig. 11 Ditopic fluorescent PET sensors **a** and **b**.

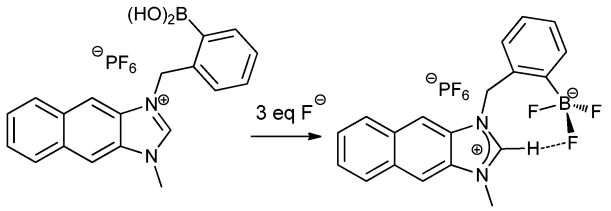
potassium fluoride and other similar ion pairs such as potassium chloride and potassium bromide.

The dynamic characteristics of this *sensor* are particularly attractive. Not only are both guests required at the binding sites to generate a fluorescence response but it is possible to add and remove individual guests producing a controlled and reversible read-out signal derived from the AND logic functionality inherent to the sensor.

The observed stability constants (K_{obs}) for sensor **20a** were 10×10^3 M^{-1} with potassium and 320 M^{-1} with fluoride. Those for sensor **20b** were 50×10^3 M^{-1} with potassium and 250 M^{-1} with fluoride in methanol.

Yoon and co-workers designed and synthesised a bidentate receptor (Scheme 7) for fluoride anions that employs a boronic acid site and an imidazolium group.[72] In a competitive aqueous solvent system (95 : 5 CH_3CN : HEPES) selectivity was achieved for fluoride over acetate and phosphate anions and a ratiomeric fluorescent response was observed. The orientation of the boronic acid group was critical, with only the *ortho* derivative (shown) displaying selectivity, while the *para* and *meta* derivatives failed to display any binding selectivity. The C–H hydrogen bond donor stabilises the binding, allowing recognition to occur in competitive media (Scheme 7). Binding as depicted was confirmed by ^{19}F NMR spectroscopy which indicated that one of the three bound fluoride anions was hydrogen bonded.

3.1.4 Boron Lewis acids as sensors for other anions. A notable exception from the earlier discussed fluoride ion sensors is compound **13** (Fig. 4) which displayed a fluorescence response to chloride anions.[53] Somewhat surprisingly given that cyanide (pK_a 9.1) is more basic than fluoride (pK_a 3.2),[73] development of cyanide anion sensors based on boron centred Lewis acids has been relatively limited. However, effective boron centred cyanide detection systems have been realised.[74]



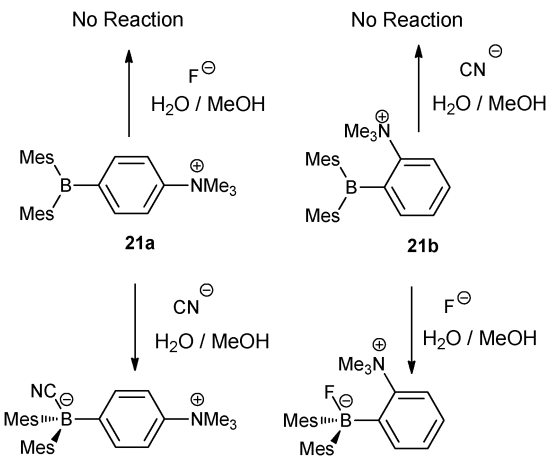
Scheme 7 A sensor that combines a boronic acid receptor with CH–anion interaction.

Perhaps the most elegant and practical demonstration of how to tip the balance in selectivity between fluoride and cyanide comes from Gabbaï,[74e] who showed that cationic boranes **21a** and **21b** (Scheme 8) are selective receptors for cyanide and fluoride respectively in water at neutral pH. The cyanide binding ability of **21a** was attributed to favorable Coulombic effects which increase the Lewis acidity of the boron centre and strengthen the receptor-cyanide interaction. The selectivity could then be switched to fluoride by positioning the trimethylammonium functionality *ortho* to the boron center in **21b**. Steric crowding around the boron centre prevents coordination of the larger cyanide anion.

Diol complexation is significantly affected by the presence of commonly employed buffer components such as phosphate, citrate and imidazole.[75] While investigating the detail of factors affecting the interaction between boronic acids and diols, it was observed that stable complexes were readily formed between boronic acids and Lewis bases. It was shown that in buffered solutions binary (boronate-Lewis base) complexes as well as ternary (boronate-Lewis base-saccharide) complexes are formed. When conducting fluorescence experiments in solutions buffered with a Lewis basic component there is a "medium dependence" related to the formation of Lewis base adducts. These complexes reduce the free boronate and boronic acid concentrations, diminishing the observed stability constants (K_{obs}).

3.2 Saccharide sensors

Whilst biological systems are able to expel water from binding pockets in order to sequester analytes, non-covalent



Scheme 8 Cyanide and Fluoride selectivity of **21a** and **21b**.

interaction based synthetic monomeric receptors where hydrogen bonding alone has been able to compete with water (solvent) for lower concentrations of monosaccharides have not been widely reported, apart from the effective examples of Davis *et al.* where hydrogen bonding receptors have been shown to be capable of binding D-glucose in water.[76]

Following the first report of boronic acid interactions of this kind by Czarnik in 1992,[77] D-glucose selectivity was achieved in 1994 by James and Shinkai.[78] A year later this was followed up by asymmetric saccharide recognition.[79] Qualitatively, binding constants (K) with monoboronic acids increased as follows: D-glucose < D-galactose < D-fructose,[12] and two receptor units were required for saccharide selectivity is to be achieved.[80]

A convenient fluorescent boronic acid unit a "click fluor" was assembled *via* a Huisgen [3 + 2] cycloaddition,[4u,81] an approach suited to a modular synthesis.[82] The so-called "click reaction"[83] forms a 1,2,3-triazole ring from an azide and a terminal alkyne which creates a fluorescent sensor from non-fluorescent constituent parts (Fig. 12). The phrase "click-fluor" describes the generation of a fluorophore from non-fluorescent constituent parts *via* a so called "click reaction". Two of the most attractive features of "click-fluor" are that a fluorophore is generated when the triazole is formed and the wide availability of acetylene units facilitating diversity.

White *et al.* showed the utility of the afforementioned *clickfluor* as a scaffold for probing reactions of boronic acids, although exploitation of the inherent sensing opportunities of the *clickfluor* construct were ignored.[84] However, a uniquely exquisite example of a reaction probe has been reported by Jiang and co-workers who used a Suzuki reaction of a boronic acid to enhance a sensing regime.[85] A catalytic Suzuki homocoupling reaction of phenyl boroinc acid was used as the probe for saccharide binding, since the rates of reaction are different for free boronic acid and saccharide-generated boronic ester the sensing read-out was the fluorescene of the generated biphenyl (Scheme 9).

Fluorescent boronic acids have also been prepared using Huisgen [3 + 2] cycloadditions by Smietana and Vasseur who employed a Seyferth-Gilbert method in a one pot process to generate alkynes from boronic acid aldehydes which were then coupled with fluorophore appended azides.[86] Wang has prepared acetylene substituted boronic acids for [3 + 2] cycloaddition[87] and suggested their use as building blocks for click reactions. Wang also used [3 + 2] cycloaddition reactions to prepare a boronic-acid-appended thymidine triphosphate for incorporation into DNA.[88] Robust electrochemically responsive sensors for saccaharides have also been developed,[89] and the interactions of boronic acids with dicarboxylic acids,[90] α-hydroxy carboxylic acids, catechol and L-dopa, dopamine, caffeic acid and alizarin red S have resulted in the development of related chemosensors.[4w,16e,33,90c,91] A recent development

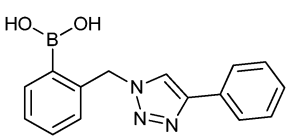
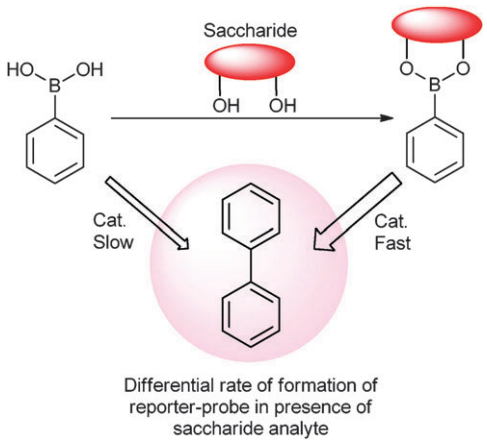


Fig. 12 The first so-called "*Click-Fluor*".



Scheme 9 Coupling catalysis to sensing to provide a uniquely sensitive saccharide reporting regime.

revealed a cation-π interaction has been revealed as a potential signalling regime for the detection of boron–diol interactions[92] and the boron–diol interaction has also been used in combination with lanthanide complexes for the recognition of sugar motifs.[93]

3.3 Dye displacement assay

A dye displacement assay is primarily a colourimetric competitive binding sensor system where an analyte displaces a dye from a receptor, this displacement results in some colour change which can be related to the amount of analyte present. Pioneering reports in this arena include the seminal protocols of Anslyn,[94] Severin[95] and Singaram[96] all of which broadly lie under the "Supramolecular Sensing" umbrella.[97]

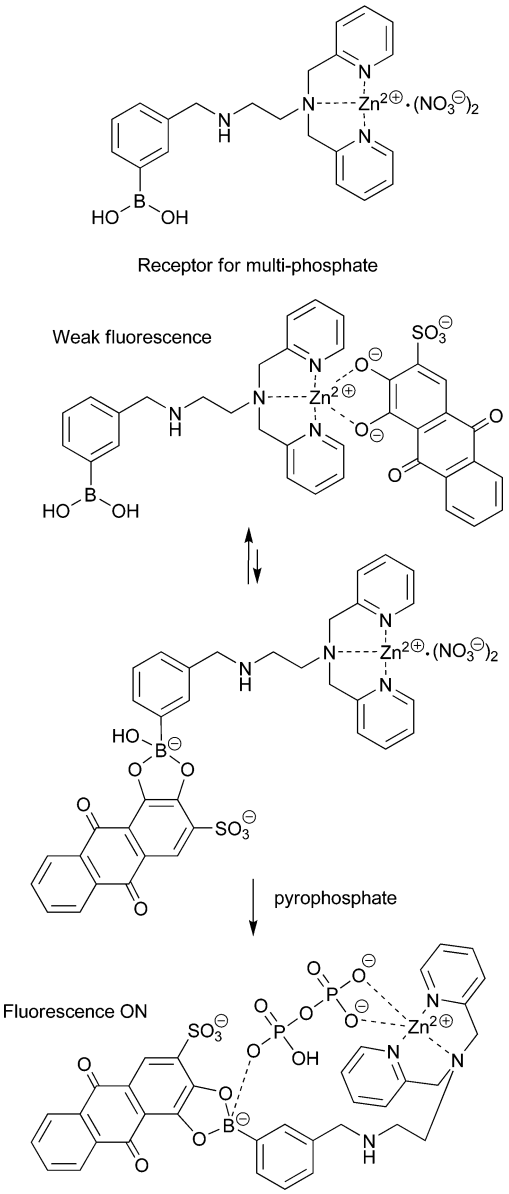
Boronic acids in combination with (1,2-diol containing) alizarin red-S (ARS) have been used in competitive binding assays.[16b,98] For example, anion mediated enhancement of alizarin red binding to boronic acids has been used to determine the presence and amount of analyte anions in a given system.[99]

The combination of a boronic acid and a zinc complex in one molecule gave rise to a pyrophosphate sensor that binds the fluorescent molecule ARS in the absences of phosphate (and displays reduced fluorescence as a result). Upon exposure to PPi ARS becomes part of a now highly fluorogenic unit, and the fluorescence increases (Scheme 10.).[99b]

A related boron–zinc system has also been developed for the detection of phosphosugar using a slightly modified dye (Scheme 11),[100] an imine containing ligand system for zinc has also found utility in the same area (Fig. 13).[101]

Freeman *et al.* detailed a fluorescence resonance energy transfer (FRET) competitive binding protocol between CdSe-ZnS quantum dots and fluorophore-labeled galactose or dopamine, boronic acid-functionalized quantum dots thus allowed optical detection of galactose, glucose, or dopamine (Scheme 12).[102] Zhou and co-workers have also reported a quantum dot boronic acid system for the detection of glucose.[103]

Anslyn and co-workers have taken the dye displacement protocol to the next level by using the dye uptake rates in combination with their peptidic array technology,[94c,104] with



Scheme 10 Pyrophosphate-induced reorganization of a reporter–receptor assembly *via* boronate esterification.

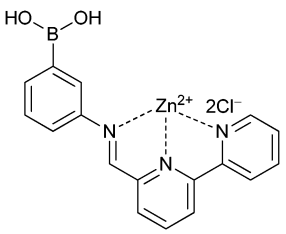
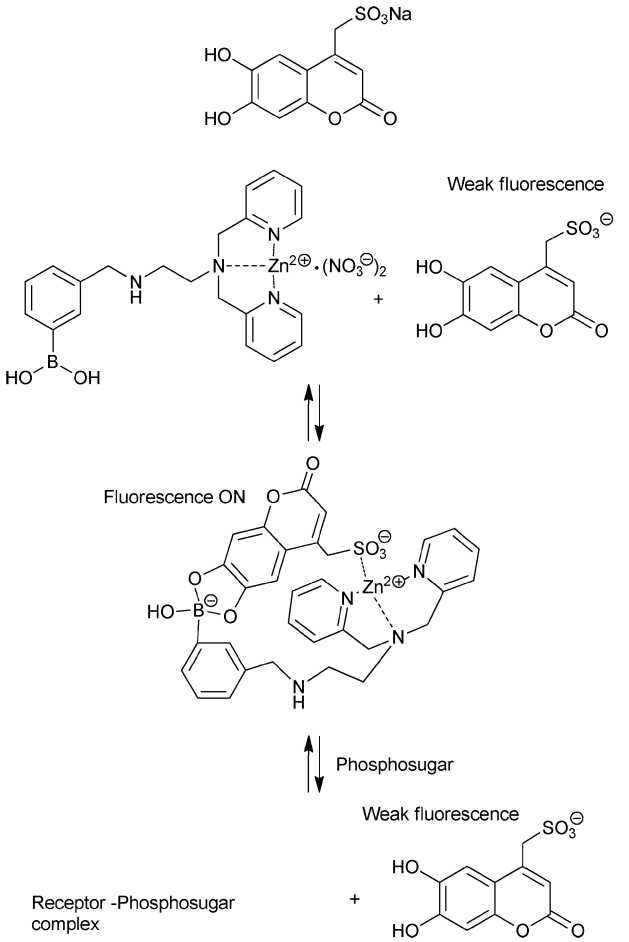


Fig. 13 Boron–zinc phosphosugar receptor.

incorporated boronic acid receptors to classify and characterise multiple diol appended analytes.[105]

Polymers containing boron are of great interest in various scenarios including electronic, optical and sensing applications, as recently reviewed by Jäkle.[106] James has also incorporated



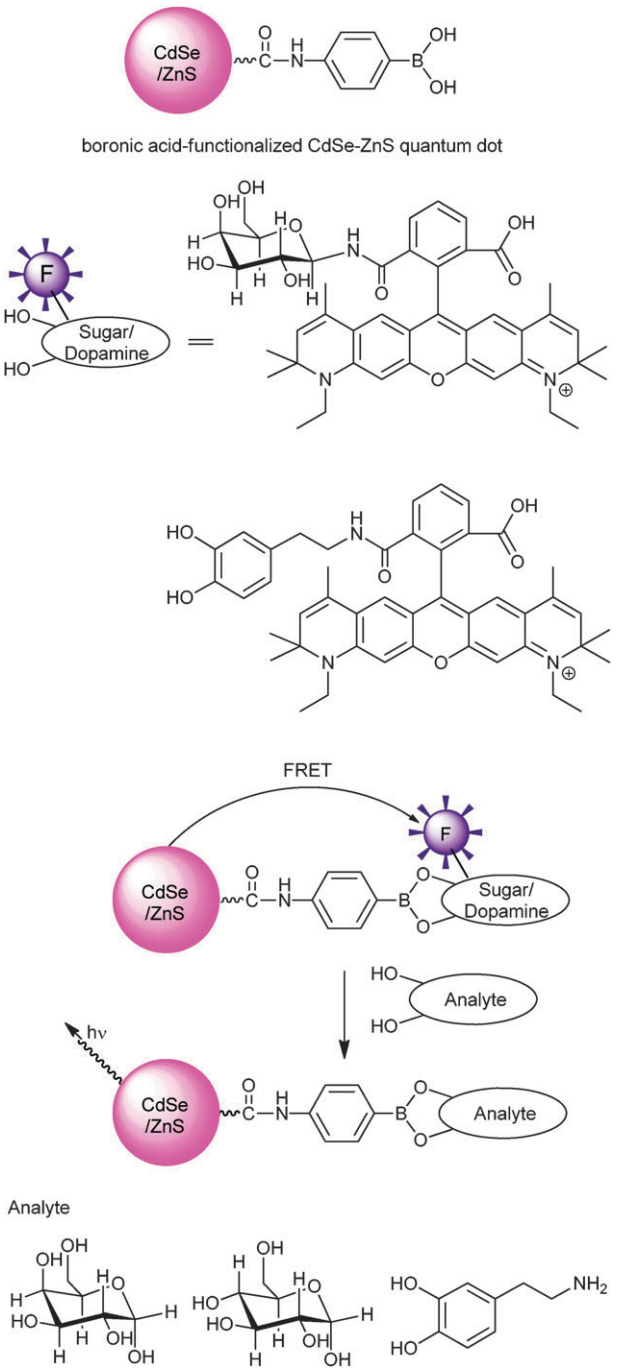
Scheme 11 Boron–zinc phosphosugar receptor used in a dye displacement assay.

modular fluorescent receptors into polymer constructs.[107] Hydrogels have found application in dye displacement assays of this kind, for example Singaram demonstrated a polyacrylamide boron pyridinium conjugate (Fig. 14).[108–110]

Elmas *et al.* detailed a temperature sensitive co-polymer which contained boronic acid units, its interaction with ARS and loss of fluorescence upon exposure to a series of analytes was discussed,[111] and Kim *et al.* described how boronic acid containing polymers can have their solubility modulated by the presence of saccharides (Scheme 13).[112]

Boronic acid containing hydrogels that had been developed for electrophoresis applications (discussed later),[113] were reasoned to be a suitable stationary phase for a similar supported system. Thus, this platform allows a dye displacement assay to be performed.[4x]

Hydrogel spheres, 5 mm in diameter, incorporating phenylboronic acid functionality were exposed to ARS dye, the corresponding boronic ester was formed. Once excess dye had been removed by washing, the spheres were exposure to an analyte diol (fructose for example) releasing the dye into solution (as represented in Scheme 14). To demonstrate the hydrogel displacement assay the relative amounts of boron binding species (saccharides) in samples of fruit juices were determined.



Scheme 12 Competitive analysis of monosaccharides or dopamine by boronic acid-functionalized CdSe–ZnS quantum dots with fluorophore-labeled galactose or fluorophore-labeled dopamine.

A hypsochromic shift is indicative of ARS binding to boron, ARS develops an orange colour on binding to boron.[99a] Comparing 10 × 10 × 1.5 mm^3 gel slabs with and without boron, Fig. 15 right and centre respectively (a boron containing gel prior to exposure to ARS is shown for comparison—left), it is possible to observe the colour difference. It is important to note that Hajizadeh *et al.* independently reported a similar system at the same time,[114] their ARS treated hydrogel was appended to glass plates and the interaction with glucose was studied.

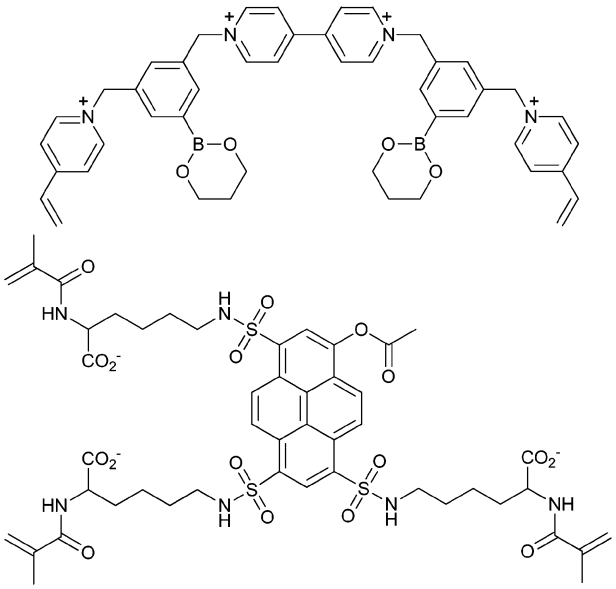
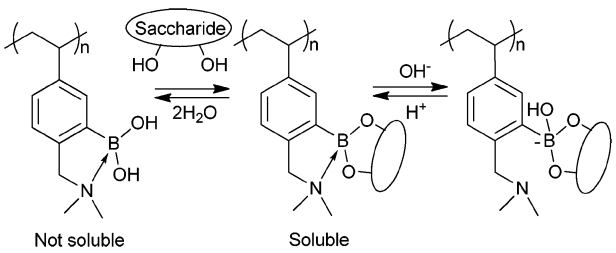
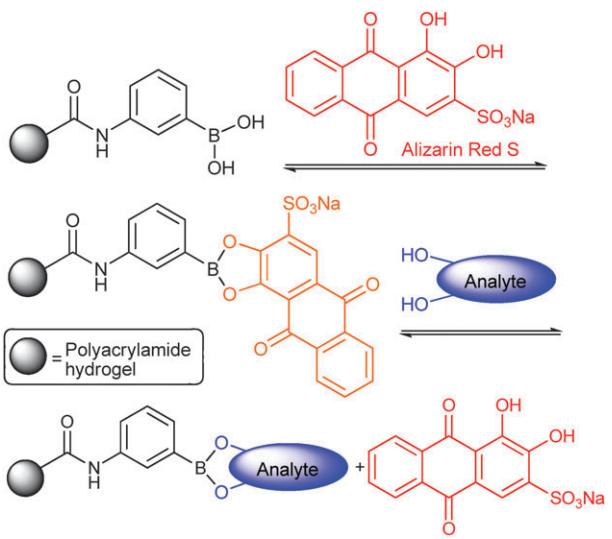


Fig. 14 Building blocks for Singaram's hydrogel boronic acid sensors.



Scheme 13 Induce polymer solubility upon saccharide binding.



Scheme 14 Binding and analyte-mediated release of alizarin red-S with hydrogel-bound boronic acid.[4x]

The interaction of ARS with phenyl boronic acid has also been used in a study where thermally responsive, glucose appended, polymers influenced bacterial aggregation.[115]

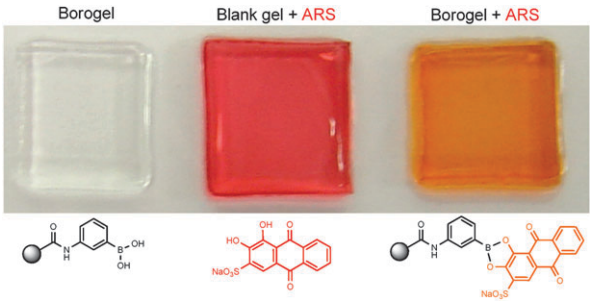


Fig. 15 Gel slabs: Borogel (left); blank gel plus alizarin red-S (middle) and borogel plus alizarin red-S (right).[4x]

3.4 Fluorophore quencher interactions

Singaram has exploited fluorophore-viologen quencher interactions in diol detection,[96a,e,b,108a,116] and it was proposed by us that the 'molecular beacon' fluorophore—quencher pairs methodology used in quantitative PCR assays such as Taqman™ may be a useful signalling regime in the study of boron diol interactions. In order to probe this hypothesis a new signalling regime was conceived whereby a fluorescent boronic acid, that when exposed to an analyte diol, appended with a quencher, would reduce the fluorescence output of the system due to the formation of a *static* fluorphore-quencher pair. Thus, signalling the presence of the diol appended quencher (Fig. 16).

A fluorescein boronic acid derivative was simply prepared from commercially available materials in order to function as the fluorescent partner and a series of methyl red inspired diols were synthesised as potential boronate ester forming quencher partners to probe a FRET quenching/sensing regime based on boronate ester formation (see Fig. 17).[4y]

A detailed study of the combination of fluorescein boronic acid with diol appended quenchers **22a–c**, and comparison with the fluorescence outputs of non-boron or non-diol containing systems (*i.e.* fluorescein or methyl red were employed directly) revealed boronate ester formation does indeed result in a quenching enhancement in each case, and that compound **22c** was the best overall quencher based on the ratiomentric quenching enhancement between the non-binding *dynamic* systems *versus* the boronate forming *static* systems.[4y]

In the same report nucleosides were also shown to bind to the same fluorescein boronic acid derivative. Whilst the

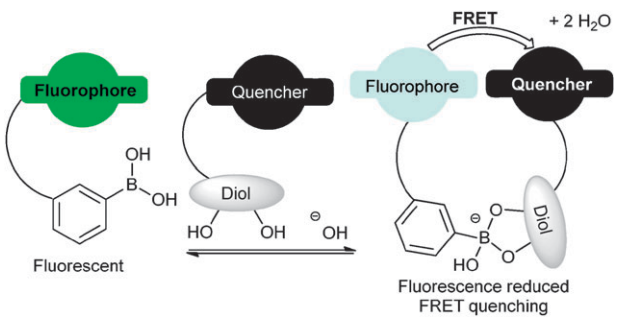


Fig. 16 A fluorophore appended boronic acid interacting with a diol-appended quencher.

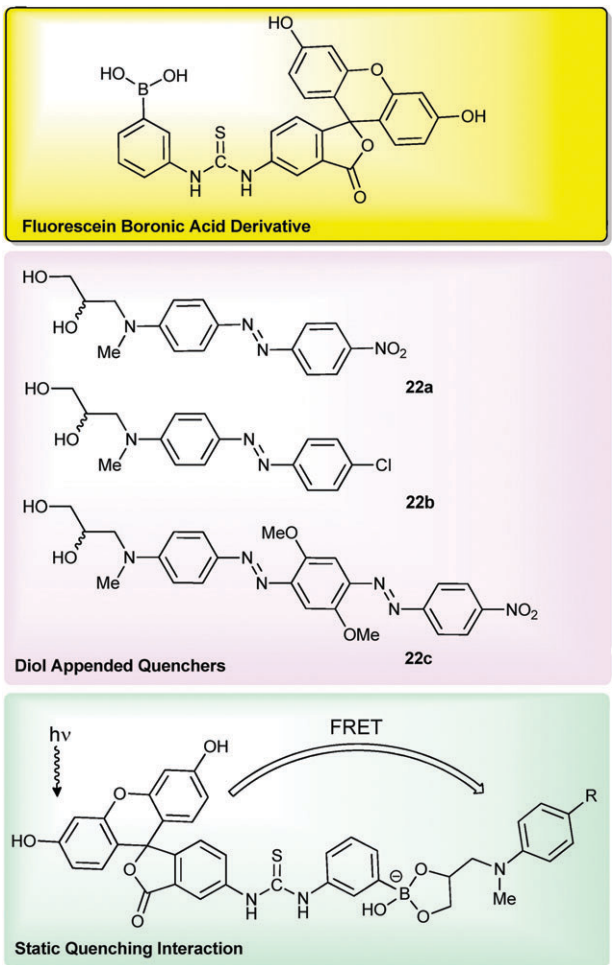


Fig. 17 A fluorescein boronic acid derivative, three diol appended quenchers (**22a–c**) and a representation of the FRET quenching interaction.

quenching ability of each nucleoside tested was different the same ratiometric quenching enhancement was observed in each case, suggesting similar binding affinities. In this light it is interesting to note that Zhong and co-workers found boronic acid appended polyethylenimine greatly enhanced gene delivery efficiency to cells.[117]

It is also noteworthy that Strongin and co-workers have utilised a bis-boronic acid derivative of fluorescein in the selective detection of saccharides (Fig. 18).[118] A motif that has also found utility in selective recognition of protein tetraserine motifs.[119]

Self assembled boronic acid hybrid systems for surface plasmon enhanced fluorecence detection of quencher labeled diols were also disclosed.[4v] As discussed in the preceding paragraph when a quencher tagged diol binds to a fluorescent boronic acid receptor the fluorescence signal is quenched, therefore "read-out" of diol binding is observed as a decrease in the total fluorescence. Surface plasmon excitation of the read-out fluorophore has two advantages, firstly SPR may be concomitantly conducted, and secondly no incident light enters the sample chamber meaning any observed photons are due only to excitation and emission from the surface

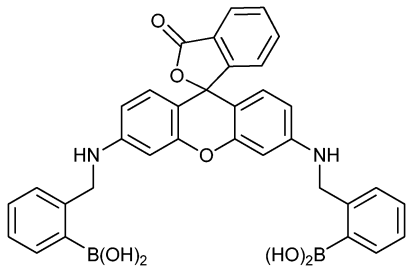


Fig. 18 Bisboronic acid derivative of fluorescein, a saccharide receptor and sensor.

appended fluorophores. Utilising a boronic acid receptor to catch quencher analytes is a generic sensing format, schematically illustrated in Fig. 19 with an underlying mechanism closely related to that of Fig. 17.

In order to assemble a sensor construct at a gold-strepatividin surface the molecule coined FLAB (Fluorophore Linker boronic Acid Biotin) was prepared. The design incorporated a terminal biotin for attachment to surface bound streptavidin, a boronic acid receptor and a fluorophore (Alexa-fluor 647, Invitrogen, ex_{max} 647 nm). Quencher-diol conjugate was prepared utilising a quencher for Alexa-Fluor 647, BHQ-3 (Biosearch Tech) (Fig. 20).[4v] Attachment of FLAB to a streptavidin appended gold surface was confirmed by both SPR and concomitant fluorescence (f-SPR). Exposure of the surface prepared to BHQ-diol gave rise to both fluorescence quenching and an SPR response demonstrating the potential for the dual techniques of SPR and fluorecence to work in unison in a sensor regime under the guise of f-SPR.

4. Boronic acids in separation science

4.1 Chromatography, analysis and separation

The reversible interaction of boronic acids with diol motifs has been exploited in separation science to great effect. Incorporation of boronic acids into the various stationary phases employed in chromatographic techniques has allowed for saccharide selective or specific separation protocols to be developed.[6e,g,16a,120] A particularly note worthy area that lies outwith the remit of this current review is the development of boron affinity columns used in HPLC. However, for more information a collection of pertinent references are provided.[6f,121]

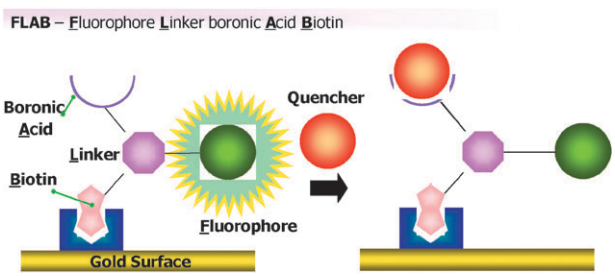


Fig. 19 Surface appended FLAB (Fluorophore Linker boronic Acid Biotin) for use in a fluorescence surface plasmon resonance (f-SPR).

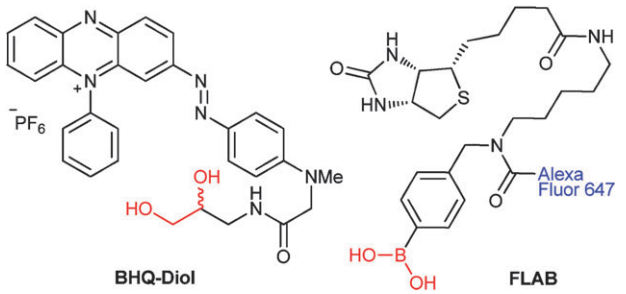


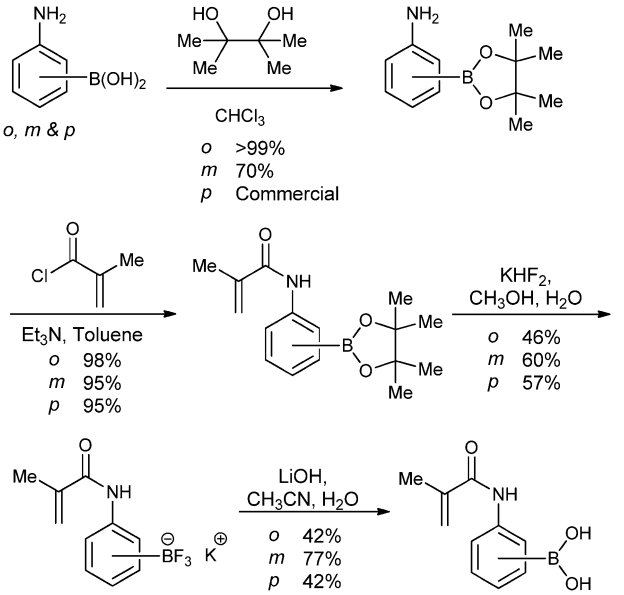
Fig. 20 BHQ-Diol and FLAB units for f-SPR assay.

4.1.1 Electrophoresis. Polyacrylamide gel electrophoresis exploits hydrogel polymers to separate molecules on a size and charge basis. Among the useful separations of biomolecules that electrophoresis is commonly employed for is a technique for the separation of carbohydrates called FACE (Fluorophore Assisted Carbohydrate Electrophoresis), which is especially notable for its ability to separate and sort oligosaccharides on a size and charge basis.[122] However the FACE technique requires the labeling of analytes with a fluorophore, and does not separate saccharides of similar size and charge particularly well and is limited to reducing sugars. Whilst protein glyco-conjugates may be visualised by staining, differing patterns of sugars in molecules of similar charge and mass can not be addressed so the technique is not applicable to the analysis of or differentiation between many glycated proteins and other sugar appended glyco-conjugates.

In order to address the need to provide a separation tool for similar mass saccharides Fossey and co-workers led the development of Boron Affinity Saccharide Electrophoresis (BASE).[113a] The method exploits the different affinities of saccharides for boronic acid which could modify gel electrophoresis mobilities to varying extents, providing boron could be incorporated into the stationary phase of an electrophoretic FACE experiment. Thus, a protocol for incorporation of boroinc acid motifs into hydrogel domains was sought. Previous examples of boronic acid saccharide sensors served as inspiration,[123] and the team synthesised a range of acrylamide boronic acids,[113b] which were readily incorporated into a polyacrylamide hydrogel matrix of the kind employed in electrophoresis.[113a]

Scheme 15 outlines the synthesis of *ortho*, *meta* and *para* methacrylamido phenylboronic acids. Pinnacol protection of aniline boronic acids is followed by conversion to the corresponding acrylamides. A significantly troublesome hydrolysis of the pinnacol protected acrylamide could be avoided by employing a two step D'Hooge acrylamido boronate deprotection. Such acrylamides are prone to self polymerisation during isolation and purification so care was taken to avoid conditions leading to polymerisation. As such initial conversion of the pinnacol esters to the highly crystalline potassium trifluoroborate salts was then followed by a mild treatment with lithium hydroxide to furnish the corresponding boronic acids. Whilst yields were still not *high* they were significantly higher than those that could be achieved by the other routes attempted.[113b]

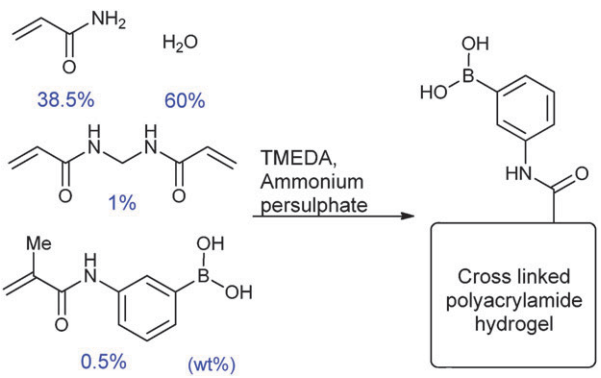
With facile routes to acrylamide boronic acids in hand boronic acid hydrogel formulation was optimised, it was



Scheme 15 Synthesis of methacrylamido boronic acids, employing the *D'Hooge Deprotection* strategy.

eventually found that whilst pinnacol deprotection was required the results were essentially the same whether boronic acid or glycol protected boronic esters were used, the two motifs were essentially interchangable under the subsequent electrophoretic conditions employed. For comparison of the existing FACE technique with the new BASE technique an optimal formulation was found to consist of 60 wt% water, 0.5 wt% boron containing monomer, 1 wt% methylene bisacrylamide (cross-linker) and 38.5% acrylamide, Scheme 16. Blank (non-boron containing) gels were prepared with 39 wt% acrylamide, all other conditions and reagents were unchanged.

Hydrogels could be prepared that contained *ortho*, *meta* and *para* boronic acids although solubility allowed up to about 3% reliable incorporation, but this was more than enough to obtain excellent results in electrophoresis. For the majority of investigations the *meta* derivative was preferred due to its overall higher synthetic yield, in comparison to the *para* derivative no differences were observed in terms of electrophoresis applications, however the *ortho* derivative gave less effective separation (analyte mobility was more facile) and was



Scheme 16 Gel formulat protocol.

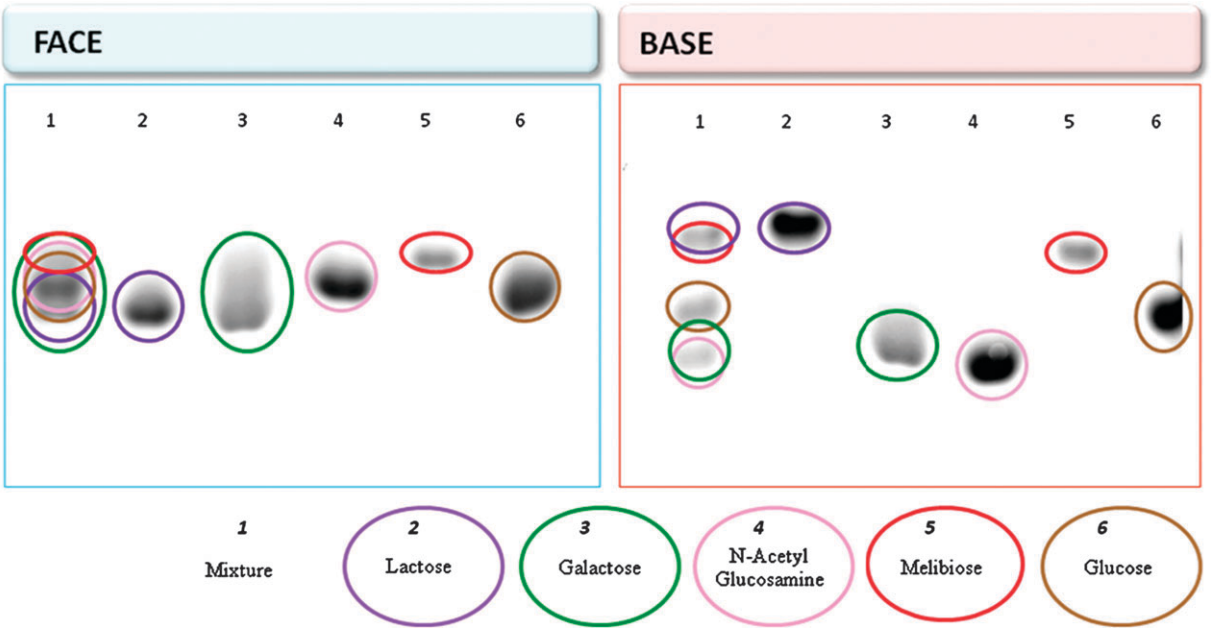


Fig. 21 Comparison of electrophoretic separation of AMAC-labelled saccharides utilising FACE and BASE (with and without boron respectively).

prone to degradation (*ortho* heteroatom assisted increase in lability of boronic ester part and self polymerisation are speculated to be the origin of less effective separation and degradation respectively).

Hand-poured gels which did and did not contain boron (BASE and FACE technique respectively) were compared for the electrophoretic separation of fluorophore[124,125] labeled saccharides. Exemplified in Fig. 21 where FACE performs poorly in separating a series of 2-AMAC labeled saccharides, the BASE system induces dramatic mobility differences among the saccharides investigated. In the BASE gel previously inseparable saccharides are now clearly resolved, even though in some cases resolution is not perfect the modulated mobility achieved as a function of reversible boron diol interactions within a hydrogel domain opened the door to a range of new applications, not only in electrophoresis,[126] but also for applications such as hydrogel sensors mentioned earlier.[4x]

BASE was next employed in the detection of a D-gluconolactone modification of a protein that had been shown by van den Elsen to inhibit the innate immune system and is under development as a therapy for complement mediated acute inflammatory diseases. The protein contains a 25-residue *N*-terminal tag (MSYHHHHHHDYDIPTTENLYFQGAM),[127] mass spectrometry analysis of similar constructs containing the same tag have been shown it to be especially prone to 6-phosphogluconoylation (6PGL). Upto this point *N*-terminal adducts had only been 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, from dephosphorylation).[128] Purified protein was exposed to gluconolactone and its electrophoretic analysis was performed at various time intervals by standard polyacrylamide gel electrophoresis (PAGE) and protein BASE (coined Pro-BASE), Fig. 22. Even after less than one minute of protein incubation with gluconolactone, Pro-BASE reveals a new band which was almost indistinguishable from the main band by a normal electrophoresis experiment. The band grew more intense over 16 h, as shown in centre left and centre right lanes in the Pro-BASE gel, Fig. 22. What appears as a small shadow to the main 16.5 kDa band in a normal experiment has an increased apparent or *virtual* molecular weight in the Pro-BASE system.

The term *virtual molecular weight* is used to describe the apparent molecular weight, against the molecular weight standard ladder (right hand lane) and the term *relative virtual molecular weight* is the relative apparent mass increase due to boron incorporation, in this case virtual molecular weight is ~60 kDa corresponding to and almost four fold increase in

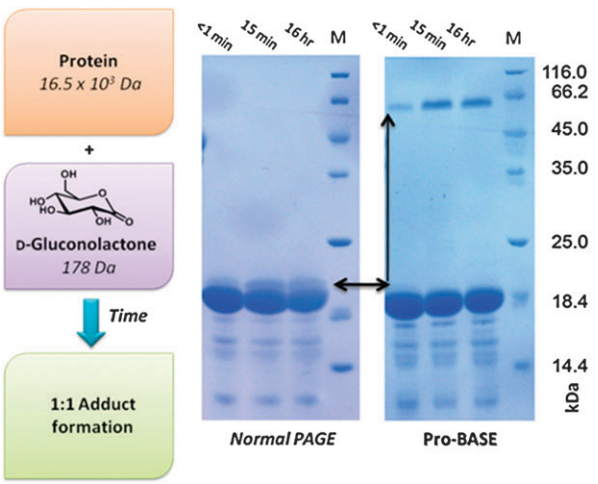


Fig. 22 Separation by protein-Boron Assisted Saccharide Electrophoresis (pro-BASE) of a glyconoylated protein utilising stationary phases that do not (left) and do (right) contain boronic acid.

apparent molecular weight. Mass spectrometry analysis strongly indicates that the new band is indeed the mono-gluconoylated adduct and further experiments over a range of boron monomer inclusion percentages, confirmed that its retention (or *virtual molecular weight*) is proportional to the boron content in the gel confirming the new band is directly modulated by boron. The team then went on to describe how glycated and glycosylated proteins (non-enzymatic and enzymatic addition of sugars to proteins respectively) could also be distinguished by this technique since the molecular construction of the linkage to the protein is different the sugar part's interaction with the boron in the gel is also different and hence separation is possible.[126a] Since glycoconjugate modification is a manifestation of numerous disease states it is envisaged that such separation regimes will have future applications in the disease diagnosis and monitoring arenas.

5. Conclusions

The reversible interaction of boronic acids is a versatile and robust regime for sensing and separation. The reversible formation of boronic esters upon exposure to diol motifs and boronate formation with anions enables recognition and signalling through a variety of mechanisms. Chromatography that utilises boron in its stationary phase provides an affinity domain for separations in a variety of settings. Coupling sensing and separation remains at the cutting edge of this field, with the backdrop of the literature surveyed here it is reasonable to predict in roads to that end will be made possible.

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