Exploring the use of APTS as a fluorescent reporter dye for continuous glucose sensing†‡

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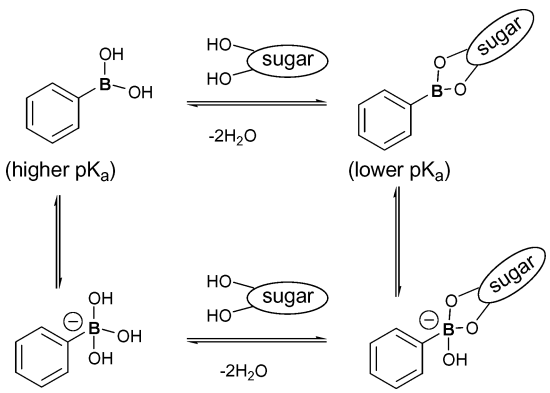
The anionic fluorescent dye, aminopyrene trisulfonic acid (APTS), was synthesized and used in a solution-based two-component glucose-sensing system comprising the dye and a boronic acid-appended viologen. The fluorescence of the dye was quenched in the presence of the viologen and the fluorescence restored upon glucose addition. An important feature of this fluorophore is that it can be covalently bonded to a polymer through the amine group without a significant effect on optical properties. Two APTS derivatives, functionalized with polymerizable groups, were synthesized and immobilized in hydroxyethyl methacrylate (HEMA)-based hydrogels. The latter were used to continuously monitor glucose. The fluorescence signal modulation, signal stability, reversibility, reproducibility, and pH sensitivity of the hydrogels were evaluated. The APTS dyes described herein are insensitive to pH changes within the physiological range, both in solution and when immobilized in a hydrogel. When APTS is used in conjunction with boronic acid-appended viologens to sense glucose, the system displays some pH sensitivity because of the presence of the boronic acid.

Introduction

The need for improved methods of glucose measurement in the treatment of diabetes has long been recognized by scientists and healthcare professionals.[1] In addition to diabetics, critically ill patients in the intensive care unit (ICU) also require improved methods of glucose detection for implementation of tight glycemic control (TGC).[2] A method that provides accurate, real-time, continuous monitoring of blood glucose levels is essential to facilitate TGC. Commercial sensors are enzyme-based. They employ enzymes such as glucose oxidase (GOX) or glucose dehydrogenase (GDH), immobilized in a polymer matrix. The enzymes function both as catalysts and as glucose receptors.[3] The glucose binding protein Concavalin A has also been used as a glucose receptor in optical sensing systems.[4] The drawbacks inherent in these approaches stimulated extensive research on alternative systems employing synthetic saccharide receptors, especially those comprising boronic acids and fluorescent dyes[5] These systems, when immobilized, offer promise as continuous glucose monitors (CGM) for *in vivo* applications.[6]

Interest in the use of boronic acid receptors[7–9] is based on the well-documented ability of arylboronic acids to reversibly bind diols,[10] including glucose, in water (Scheme 1).

Research in our laboratory has focused on the development of a two-component, fluorescence-based saccharide-sensing system that utilizes boronic acids as glucose receptors.[11] The system comprises a fluorescent anionic dye and a boronic acid-appended cationic benzyl viologen. In the absence of glucose, the viologen moiety quenches the fluorescence of the dye; it becomes a far less effective quencher when bound to glucose. Thus, the measurable fluorescence of the system is modulated in response to varying glucose concentration. Using this combination, we have demonstrated continuous and reversible glucose sensing *in vitro* by immobilizing the sensing elements in a thin-film poly(2-hydroxyethyl methacrylate) (p(HEMA)) hydrogel.[12] These studies utilized polymerizable derivatives of hydroxypyrene trisulfonic acid (HPTS) (Fig. 1). HPTS-based dyes can be excited at 460 nm and emit around 510 nm, which is advantageous for use in sensors. Even though they perform remarkably well for glucose sensing at pH 7.4, the fact that they are pH sensitive in the physiologically relevant range (in the ICU, the pH of a patient's blood can vary between 6.8 and 7.8)[13] warranted a search for dyes that are less pH



Scheme 1 Equilibria between phenyl boronic acid and a generic diol in aqueous media.[10]

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† Dedicated to Professor Seiji Shinkai on the occasion of his 65th birthday.

‡ Electronic supplementary information (ESI) available: Fluorescence profiles with varying pH for MABP with APTS and HPTS; excitation and emission spectra of APTS and polymerizable derivatives; stability of hydrogels **4** and **5**; NMR spectra of APTS, APTS-BuMA, and APTS-DEGMA. See DOI: 10.1039/b821934f

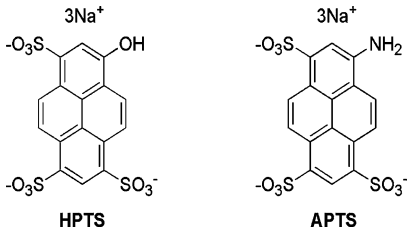


Fig. 1 Structures of water-soluble, visibly excited dyes, HPTS and APTS.

sensitive in this range. This search led us to aminopyrene trisulfonic acid (APTS) (Fig. 1).

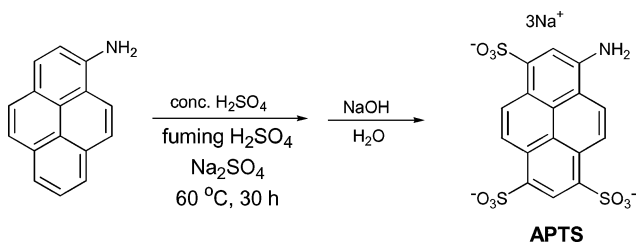
APTS has excitation and emission maxima similar to those of HPTS. The hydroxyl group on the pyrene ring in HPTS is responsible for pH sensitivity. Since the substituent on APTS is an amino group, we anticipated that APTS would only be affected at low pH, and remain pH insensitive in the physiological range.

APTS has been used to form saccharide-dye conjugates by reductive amination.[14] We reasoned that this chemistry could be utilized to synthesize a polymerizable derivative of APTS. There is an advantage in using APTS rather than HPTS for making dyes that can be immobilized into hydrogels, in that the former can be easily appended with only one polymerizable group by coupling through the amino group. In contrast, HPTS cannot be functionalized by attaching a polymerizable group through the phenolic oxygen *via* ester or ether formation because the photophysical properties of the dye are drastically affected. The excitation maximum, for example, is shifted from 460 nm to 400 nm.[15] Instead, it must be functionalized through the three sulfonic acid groups making mono-substitution difficult. As a consequence, most of the HPTS derivatives that have been synthesized are substituted with three polymerizable groups. We reasoned that a single point of attachment of the dye is preferable to multiple bonding because it would allow greater mobility in the hydrogel.[12] An added advantage is that the mono-functional dye does not participate in the cross-linking of the hydrogel.

In this paper we report the synthesis and characterization of APTS and the use of this dye in a soluble glucose-sensing ensemble. We also report the synthesis of two polymerizable APTS derivatives, the incorporation of the latter in thin-film hydrogels and the evaluation of the gels as glucose sensors.

Results and discussion

APTS was synthesized by sulfonation of aminopyrene followed by neutralization with sodium hydroxide (Scheme 2).[16]



Scheme 2 Synthesis of APTS from 1-aminopyrene.

This allowed for the preparation of gram quantities of the dye in analytically pure form. APTS was characterized in pH 7.4 phosphate buffer giving an excitation maximum at 425 nm and an emission maximum at 503 nm (Fig. 2). That the dye can be excited in the visible region and has a fairly large Stokes shift (78 nm) is advantageous for sensor applications.

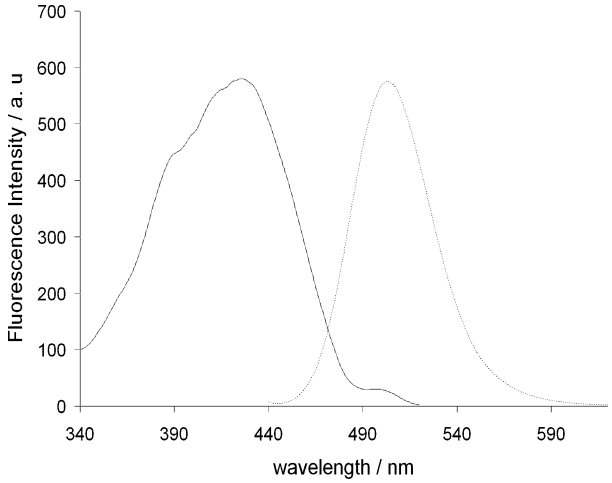


Fig. 2 Excitation and emission spectra of APTS (λ_{ex} = 425 nm, λ_{em} = 503 nm) 4×10^{-6} M in pH 7.4 phosphate buffer.

Fluorescence quenching studies

Benzyl viologens are known to effectively quench the fluorescence of anionic dyes, such as HPTS in solution.[17] Modification of benzyl viologen-type quenchers with polymerizable groups has permitted their immobilization in a polymer matrix and subsequent use in a glucose-sensing system. For example, MABP and 1MABP have been found to be effective fluorescence quenchers (Fig. 3).

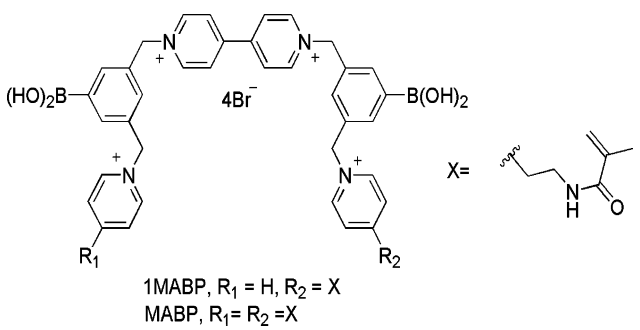


Fig. 3 Polymerizable quenchers MABP and 1MABP.

Fluorescence intensity measurements show that these quenchers respond to glucose when immobilized in a hydrogel.[12] Both MABP and 1MABP were used in this study to evaluate the performance of the APTS derivatives in solution and in thin film hydrogels. The ability of MABP to quench the fluorescence of APTS and HPTS was evaluated in pH 7.4 phosphate buffer (Fig. 4).

The Stern–Volmer quenching constants were determined to be K_s = 51,400 M^{-1} and V = 2,140 M^{-1} for APTS compared to K_s = 165,300 M^{-1} and V = 5,690 M^{-1} for HPTS.

The fluorescence of APTS is clearly quenched, albeit with a smaller quenching constant relative to HPTS. In part, this may be a consequence of differences in coulombic attraction. We have shown previously that quencher–dye interaction correlates with

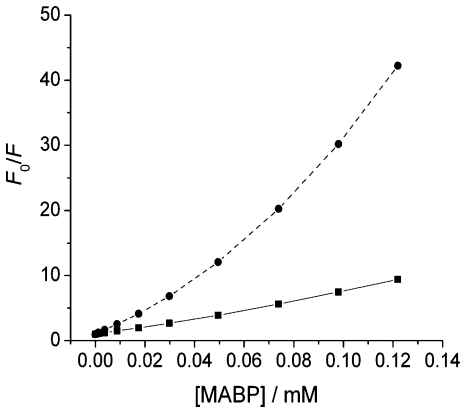


Fig. 4 Stern–Volmer plot of 4×10^{-6} M APTS (■) and HPTS (●) with increasing concentrations of MABP.

the negative charge on the dye.[15] At pH 7.4, the hydroxy group on HPTS is partially ionized, increasing the number of negative charges on the dye to greater than three. In contrast, APTS has only three negative charges at pH 7.4.

HPTS is known to form a ground state complex with viologen-type quenchers, including MABP, and this is reflected in the static quenching constant. In order to determine if APTS shows similar behavior, the formation of a ground-state complex between MABP and APTS was studied spectroscopically (Fig. 5).

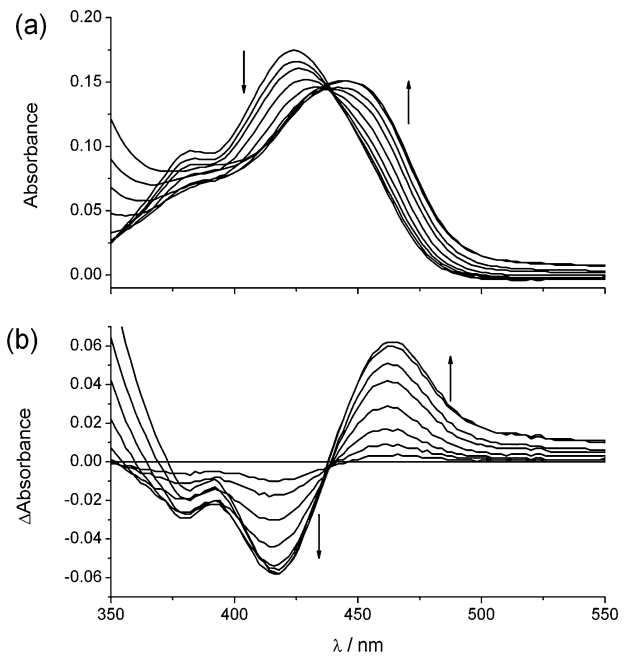


Fig. 5 (a) Absorbance spectrum of APTS alone (λ_{max} 424 nm) and with increasing concentrations of MABP. (b) Difference spectra showing the new peak appearing at 464 nm as the quencher–dye complex forms. [APTS] = 10 μM in phosphate buffer (pH 7.4, 39 mM). MABP was added with increasing concentrations of 0, 5, 10, 20, 40, 100, 150, 200, and 300 μM.

The absorbance spectrum of the dye is red-shifted upon addition of the quencher, indicating formation of a quencher–dye complex.

pH Sensitivity

A potential benefit of using APTS as a reporter dye is its lack of pH sensitivity. We verified this by measuring the fluorescence of APTS as a function of pH; the emission maximum remains essentially constant over the broad pH range of 4–10 (Fig. 6).

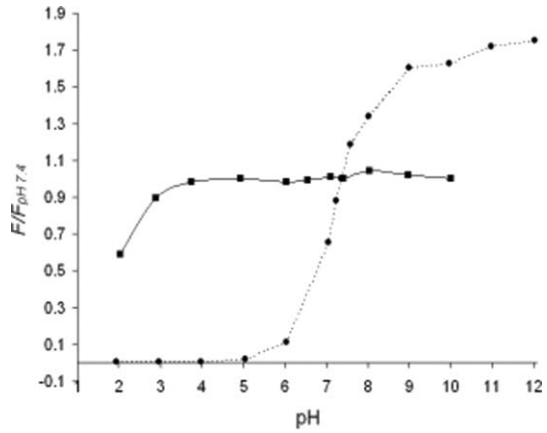


Fig. 6 Normalized fluorescence intensity of APTS (■) and HPTS (●) (4×10^{-6} M in phosphate buffer) as a function of pH. The pH was adjusted with either HCl or NaOH. The fluorescence is normalized relative to fluorescence at pH 7.4.

At pH less than 4, the fluorescence intensity drops markedly as the amine group becomes protonated. Fortunately this pH is well below the physiological pH, suggesting that APTS derivatives could be used as a component in a CGM and would not be affected by variations in pH. In contrast, HPTS is very sensitive to pH in the physiological range, and for this reason can be used as a pH indicator.[18]

Glucose-sensing studies in solution

The glucose response of quencher–dye systems in pH 7.4 PBS was compared. APTS and HPTS were each used with the quencher MABP to sense glucose in aqueous solution. One of the advantages of a two-component system over a one-component system is the ability to alter the quencher–dye ratio. The optimum quencher–dye ratio [Q/D] has been determined for many different combinations of quenchers and dyes.[17a] We chose a Q/D of 10:1 to compare the dyes in solution so that it would match the ratios used in our hydrogel studies. At this ratio, APTS shows a fluorescence increase (F/F_0) of 1.25 between 0–20 mM glucose whereas for HPTS it is 1.60. The combination of MABP:APTS has an apparent glucose binding constant of 701 M^{-1} *versus* 289 M^{-1} for MABP:HPTS. The increased binding constant for MABP:APTS could explain the diminished response compared to MABP:HPTS. Nevertheless, these results indicate that APTS is a suitable fluorophore to use in the two-component glucose-sensing system (Fig. 7).

The sensitivity of the quencher–dye systems (MABP/APTS and MABP/HPTS) to pH changes within the range of 6.8–7.8 in the presence of glucose (10 mM) was determined (see ESI‡).

In this pH range, the system utilizing APTS as the fluorescent reporter is less sensitive to changes in pH than the HPTS-containing system. The HPTS system shows a 130% increase in fluorescence intensity when the pH increases from 6.8 to 7.8. In

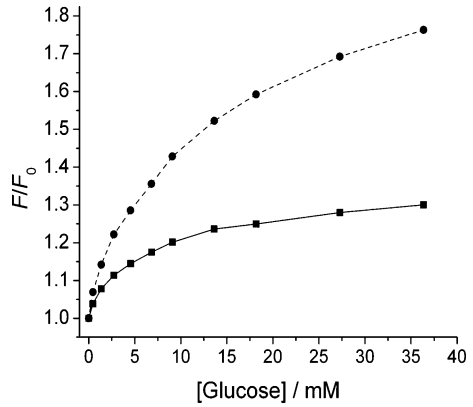


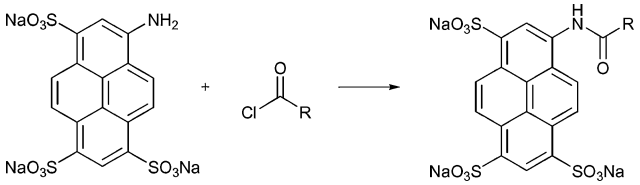
Fig. 7 Glucose-sensing of 4×10^{-6} M APTS (■) and HPTS (●) with MABP (10:1 quencher–dye ratio) with increasing amounts of glucose.

contrast, the APTS system displays only a 30% increase over that same range. Since the change in fluorescence seen in the MABP/APTS system is not caused by the dye, it must be a result of the inherent pH sensitivity of the boronic acid substituents on the quencher.[19] Nevertheless, relative to HPTS, APTS greatly decreases the pH sensitivity of the quencher–dye combination.

Synthesis of polymerizable APTS derivatives

Solution studies showed that APTS would be a suitable fluorescent reporter dye for our two-component glucose-sensing system. Although APTS works well in solution, to be useful in a continuous glucose sensing device, it must be immobilized. Our approach is to covalently bond dye and quencher to a hydrogel polymer. Consequently, we have developed synthetic routes for the preparation of polymerizable APTS derivatives. We had previously synthesized polymerizable HPTS derivatives with three polymerizable groups attached to the pyrene ring through the sulfonic acid moieties.[12] Even though APTS could also be functionalized in a similar manner, we envisioned that a single polymerizable group would result in greater mobility for the dye. This would allow better ionic interaction between dye and quencher and possibly greater signal modulation upon glucose binding. The amino functionality on APTS serves as a reactive site for attaching a single polymerizable group to the dye.

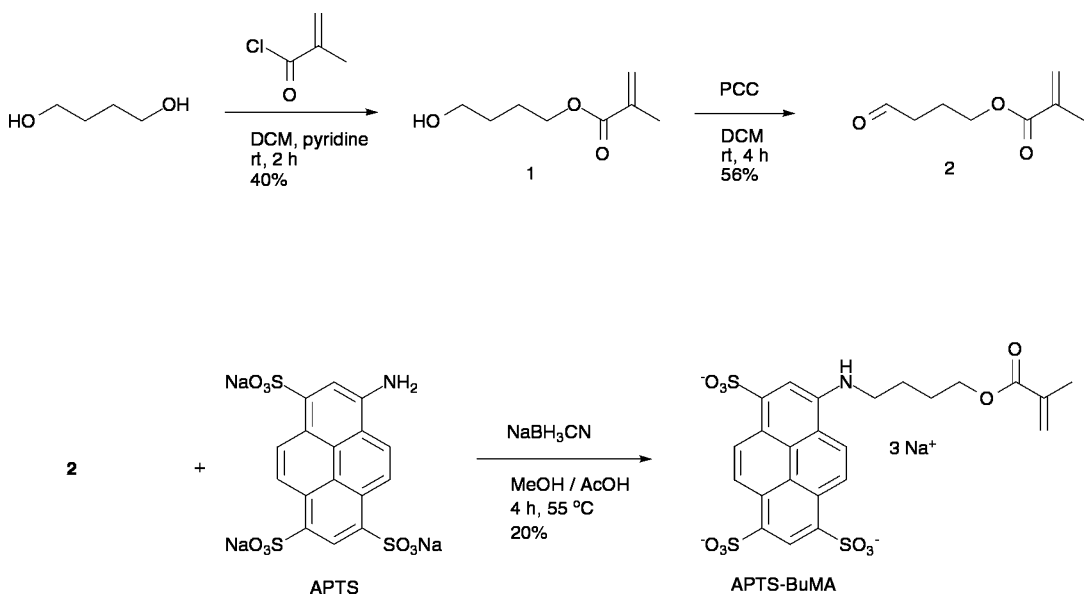
Initial synthetic efforts were focused on the much simpler method of connecting the polymerizable group through an amide bond (Scheme 3). However, this resulted in a derivative with a drastically reduced fluorescence intensity, indicating that the interaction of the free amine group with the pyrene ring is essential for the fluorescence properties of APTS.



Scheme 3 General scheme for converting APTS to an amide derivative.

We then shifted our attention to attaching an aldehyde functionalized monomer to APTS by reductive amination, assuming that the alkylated amine functionality would not significantly change the fluorescence properties. This approach was successfully used to prepare two APTS derivatives, one tethered with butylmethacrylate (APTS-BuMA) and one with diethyleneglycol methacrylate (APTS-DEGMA). APTS-BuMA was synthesized as follows (Scheme 4).

Mono-esterification of 1,4-butanediol with methacryloyl chloride gave alcohol **1**, which was oxidized with pyridinium chlorochromate (PCC) to afford aldehyde **2**. Reaction of **2** with APTS gave the imine *in situ* that was reduced with sodium cyanoborohydride to give the desired product, APTS-BuMA, a mono-functionalized anionic fluorescent dye with a methacrylate polymerizable group.



Scheme 4 Synthesis of the polymerizable dye APTS-BuMA.

For comparison, another mono-functionalized APTS dye, APTS-DEGMA, was synthesized with a linking group that is slightly longer and more hydrophilic. It was made from commercially available diethyleneglycol monomethacrylate (DEGMA). The synthetic route was similar to that used for APTS-BuMA (Scheme 5).

Spectral properties of polymerizable APTS derivatives

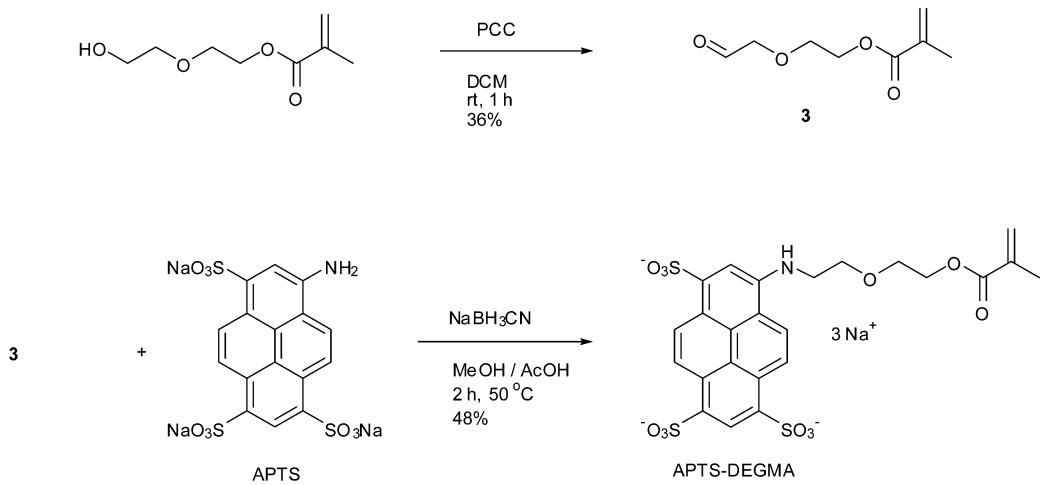
The excitation and emission spectra of both APTS monomers in pH 7.4 buffer were recorded and the optical properties of the new dyes and the parent APTS dye compared (see ESI‡). Alkylation of the amine on APTS resulted in a red-shift of both the excitation and emission maxima for APTS-DEGMA and APTS-BuMA. Both dyes had very similar bathochromic shifts with APTS-BuMA being slightly more red-shifted.

Hydrogel studies

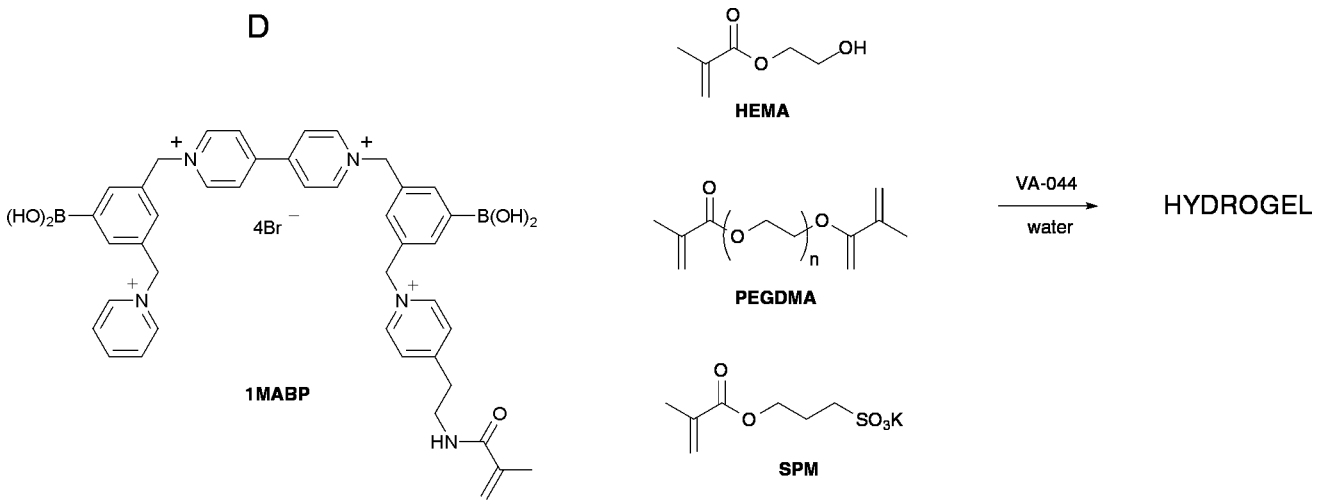
The hydrogels used in this study are cross-linked HEMA copolymers containing more than 50% water.[20] To verify incorporation of the dye monomers into the hydrogel, two dye-containing gels were prepared, **4** with APTS-BuMA and **5** with APTS-DEGMA. The APTS monomers were copolymerized with 2-hydroxyethyl methacrylate (HEMA), polyethylene glycol (1000) dimethacrylate (PEGDMA), 3-sulfopropyl methacrylate potassium salt (SPM), and 1MABP to form a hydrogels. The thermally activated radical initiator, 2,2′-azobis[2-(2-imidazolin-2-yl)propane] dihydrochloride (VA-044), was used to induce polymerization (Scheme 6).

The hydrophilic anionic co-monomer SPM was used to enhance the swelling capacity of the gels.[21] To verify that the fluorescent dyes remained immobilized within the HEMA hydrogel matrices, they were monitored for up to 24 hours while immersed in circulating pH 7.4 phosphate buffer maintained at 60 °C. They were subjected to the high temperature to ensure there was no leaching of the dyes from the hydrogels. The emission intensity remained essentially constant over the duration of the experiment, leading us to conclude that the two polymerizable dyes were successfully bonded to the polymer matrix (see ESI‡).

The APTS-DEGMA hydrogel **5**, was then tested for its sensitivity to a pH change from 6 to 8. The emission of this gel remained nearly constant over this range. In comparison, hydrogel



Scheme 5 Synthesis of the polymerizable dye APTS-DEGMA.



Scheme 6 Polymerization of APTS-derived monomers to form glucose-sensing hydrogels. Hydrogels contain the following dye (**D**) and quencher components: APTS-BuMA (**4**), APTS-DEGMA (**5**), HPTS-Lys-MA (**6**), APTS-BuMA and 1MABP (**7**), APTS-DEGMA and 1MABP (**8** and **9**).

6, prepared from polymerizable HPTS derivative HPTS-Lys-MA,[12] was highly sensitive to changes in pH within this range (Fig. 8).

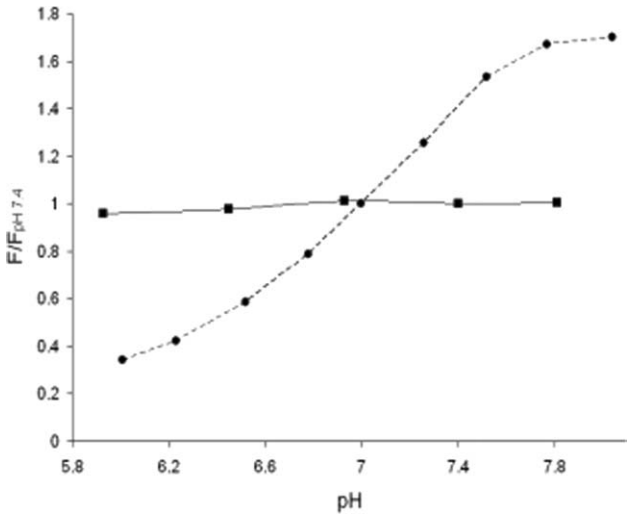


Fig. 8 Comparison of APTS-DEGMA dye-only hydrogel **5** (■) with an HPTS-Lys-MA dye-only hydrogel **6** (●) at varying pH. The fluorescence is normalized relative to intensity at pH 7.4.

To evaluate their ability to act as glucose-sensing materials, each dye monomer was combined with the quencher, 1MABP, and immobilized in a hydrogel by copolymerization. Three hydrogels, **7**, **8**, and **9** were synthesized (Scheme 6). Hydrogel **7**, which contained the dye APTS-BuMA, was made with a Q/D of 10:1 It was polymerized at 45 °C for 24 hours. After polymerization, the gel was allowed to swell in phosphate buffer for 24 hours. It was then mounted in a flow cell[12] and monitored at 528 nm (λ_{ex} = 465 nm). After allowing the gel to equilibrate in the buffer, glucose was added to the circulating stream incrementally (2.5, 5.0, 10, and 20 mM) and the change in intensity monitored. An increase in fluorescence intensity was observed that was directly related to the glucose concentration, with F/F_0 reaching 1.7 at 20 mM glucose (Fig. 9). The average response time for this gel, measured as the time required to reach 95% signal for each analyte concentration, was 0.66 h.

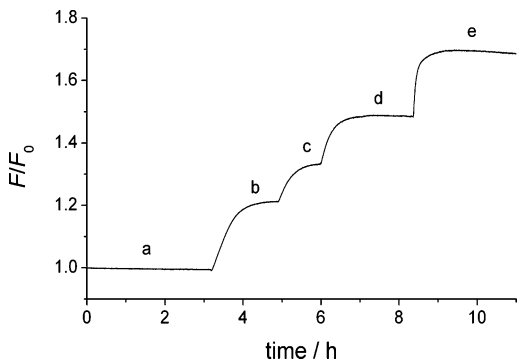


Fig. 9 Glucose response of hydrogel **7** monitored in a flow cell (λ_{ex} = 465 nm, λ_{em} = 528 nm). a) buffer, b) 2.5 mM glu, c) 5 mM glu, d) 10 mM glu, e) 20 mM glu.

Hydrogel **8** was made in a similar manner using APTS-DEGMA (Q/D = 10:1) and was subjected to different concentrations of glucose. For this gel, F/F_0 = 2.8 at 20 mM. The signal at 20 mM remained stable for 15 hours. The increased glucose modulation compared to that of **7** could be a result of the longer, more hydrophilic tether connecting the dye moiety to the polymer matrix. The plot also demonstrates the reversibility of the sensor. Decreasing the glucose concentration incrementally to zero (buffer only) brought the signal back to the starting value (Fig. 10). Average response times for this gel were 1.2 h for increasing analyte and 2.0 h for decreasing analyte.

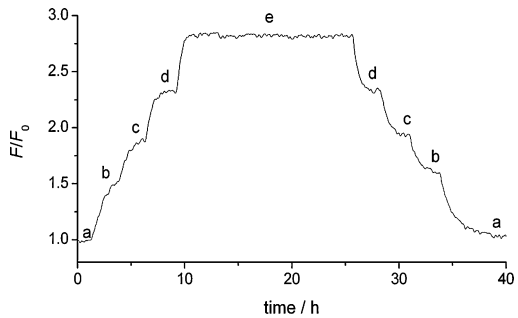


Fig. 10 Glucose response of hydrogel **8** monitored in a flow cell (λ_{ex} = 465 nm, λ_{em} = 528 nm). a) buffer, b) 2.5 mM glu, c) 5 mM glu, d) 10 mM glu, e) 20 mM glu.

Another hydrogel (**9**) was prepared with 1MABP and APTS-DEGMA (Q/D = 30:1). The reproducibility of the glucose response was tested by repeatedly exposing this gel to 20 mM of glucose and returning to buffer (Fig. 11).

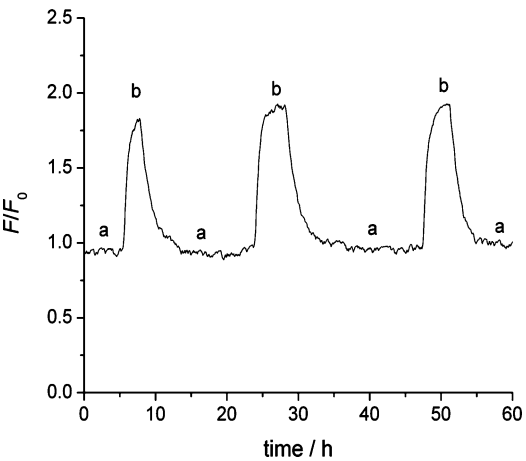


Fig. 11 Reproducibility of hydrogel **9**. a) buffer, b) 20 mM glucose.

The glucose modulation F/F_0 averaged 1.9 for the three runs over a total period of 55 h. This demonstrates that the signal is sufficiently reproducible for this system to be used as a practical glucose sensor. The average response time for this gel was 1.6 h. It should be noted that these gels were prepared with a thickness of 0.25 cm to prevent tearing during transfer and handling. Thinner gels have more rapid response times. The decreased glucose response of hydrogel **9** *versus* hydrogel **8** is probably caused by the change in Q/D. At 30:1 Q/D the fluorescence of APTS-DEGMA is quenched much better than at 10:1. Nevertheless, the gel still functions as a glucose sensor with excellent reproducibility. Of the gels tested, hydrogel **8** gave the highest signal modulation.

Conclusion

The water-soluble, anionic fluorescent dye, aminopyrene trisulfonic acid (APTS), has some photophysical properties similar to those of the commonly used fluorescent indicator, HPTS, but its sensitivity to pH is dramatically different. In particular, the fluorescence intensity of APTS remains nearly constant over a pH range from 4 to 10. This dye was used to make a solution-based, two-component fluorescent probe comprising the dye as reporter and a boronic acid-appended viologen as glucose receptor. The fluorescence of the dye was quenched in the presence of the viologen and the fluorescence restored upon glucose addition. The performance of this system nearly equals that of an HPTS-based probe, while the emission intensity of the quencher–APTS combination is much less pH sensitive. Consequently, APTS should prove useful as a reporter in our saccharide recognition system.[22]

Two APTS-based dye monomers were synthesized and incorporated into a glucose-sensing hydrogel. APTS is readily functionalized with polymerizable groups by reductive amination with only a minor effect on optical properties. The dyes retain their fluorescence characteristics when immobilized in a hydrogel by copolymerization. The present study shows further that hydrogels made with polymerizable APTS derivatives and viologen boronic acid quenchers have good modulation over the physiological range of 2.5–20 mM glucose. The signal is reproducible, reversible, and stable over the time period studied. Since the only pH responsive component in the hydrogel is the boronic acid receptor, the glucose response is likely to be less sensitive to pH changes than that of hydrogels made with HPTS derivatives. We conclude that these dyes form an important new family of fluorophores that can be used both in solution-phase probes and in polymer-based sensors.

Experimental section

Materials and methods

Reagents were used without further purification unless noted. The dyes, 8-hydroxypyrene trisulfonate trisodium salt (HPTS, pyranine) and aminopyrene, and the intermediates, 1,4-butane diol, methacryloyl chloride, diethyleneglycol monomethacrylate. and the mono-saccharide, D-glucose, were purchased from Aldrich. The preparation of the components, HPTS-LysMA, MABP, and 1MABP, was reported previously.[12] The synthetic intermediates (**1**, **2**, and **3**) used in the preparation of polymerizable APTS dyes were characterized by NMR and used without further purification. Spectra of purified polymerizable dyes can be found in the ESI.‡ All solvents were reagent grade. All aqueous solutions were prepared with water that was purified *via* a Barnstead NANOpure system (17.7 MΩ/cm). Phosphate buffers (0.1 ionic strength) were freshly prepared before use (pH 7.4: KH_2PO_4, $NaHPO_4$). Glucose solutions were freshly prepared in 0.1 ionic strength pH 7.4 phosphate buffer. The dye HPTS is sensitive to pH changes and thus pH was kept with in ± 0.02 of pH 7.40 unless otherwise noted.

Instrumentation

^{1}H-NMR spectra were recorded on a Varian 500 MHz instrument. Mass spec data was collected on a Mariner Biospectrometry workstation. Measurement of pH was carried out using a Bruker 989 pH meter. UV-VIS spectra were recorded on Shimadzu UV-1800 spectrophotometer using standard quartz cuvettes. Fluorescence spectra were recorded on a Perkin-Elmer LS50-B luminescence spectrometer. Standard quartz fluorescence cuvettes were used in all studies. APTS was excited at 424 nm. HPTS was excited at 460 nm. The emission spectra were collected from 460–650 nm. All studies were carried out under ambient conditions (25 °C, in air) unless otherwise noted. For titration experiments the added volume did not exceed 2% of the total volume for all fluorescence measurements. Solution and hydrogel measurements were made after the signal had stabilized for at least 5 minutes.

Synthesis

Aminopyrene trisulfonic acid trisodium salt (APTS). In an oven-dried 100 mL round bottom flask equipped with a drying tube (charged with drierite and NaOH pellets) was added Na_2SO_4 (8 mmol, 1.13 g) and conc. H_2SO_4 (5 mL). Aminopyrene (2 mmol, 434 mg) was added followed by fuming H_2SO_4 (20%, 6 mL) and the slurry was stirred at 60 °C for 30 h. The mixture was carefully poured into 50 mL of water and neutralized with 50% NaOH solution to pH 7 (required about 12 g NaOH). The solution was concentrated *in vacuo* and extracted with MeOH (*via* sonication) and the solids removed *via* filtration. Concentration of the MeOH solution gave 1.04 g (99% yield) of a brown powder that gave only one spot by TLC. Further purification through a plug of silica gel (NH_4OH: IPA, 1:2) yielded an orange powder. Yield: 1.04 g (99%). UV–VIS (water) λ_{max} 424 nm ($\varepsilon = 20{,}600\ M^{-1}cm^{-1}$), 383 nm. Fluorescence (pH 7.4 buffer) excitation maximum 428 nm, emission maximum 500 nm. ^{1}H NMR (CD_3OD, 500 MHz) δ 8.17 (s, 1H), 8.42 (d, J = 9.5 Hz, 1H), 8.90 (d, J = 9.5 Hz, 1H), 9.03 (d, J = 9.5 Hz, 1H), 9.13 (d, J = 9.5 Hz, 1H), 9.28 (s, 1H); ^{13}C NMR (CD_3OD, 125 MHz) δ 113.39, 116.20, 117.88, 120.56, 122.78, 123.42, 124.61, 126.31, 126.56, 127.43, 129.96, 130.48, 134.62, 135.00, 140.90, 144.48.

4-Hydroxybutyl methacrylate (1). Methacryloyl chloride (5.86 mL, 60 mmol) was added dropwise to a cooled (0 °C) solution of 1,4-butanediol (5.33 mL, 60 mmol) and pyridine (30 mL) in dichloromethane (30 mL). The reaction was stirred at room temperature for 2 h, quenched with 1M HCl (50 mL), and extracted with dichloromethane (3 × 100 mL). The combined DCM layers were washed with 3M HCl (3 × 100 mL), dried with magnesium sulfate, and evaporated to an oil which was chromatographed on silica gel using hexanes/ethyl acetate (6:4) to give 3.7 g (40% yield) of clear oil. Yield: 3.7 g (40%) ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.70 (m, 4H), 1.92 (s, 3H), 3.67 (br s, 2H), 4.17 (t, J = 6.5 Hz, 2H), 5.54 (s, 1H), 6.08 (s, 1H); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 18.31, 25.12, 29.15, 62.22, 64.54, 125.45, 136.37, 167.59.

3-Formylpropyl methacrylate (2). A solution of 1 (3.7 g, 23 mmol) in dichloromethane (10mL) was added to a suspension of pyridinium chlorochromate (7.4 g, 34.5 mmol) and celite (5 g) in dichloromethane (30 mL). The reaction was stirred at room temperature for 4 h. Diethylether (200 mL) was added and the reaction was filtered through celite. The dark brown filtrate was evaporated to a black oil which was then chromatographed on silica gel using 100% dichloromethane to yield 2.0 g (56% yield)

of clear oil. Yield 2.0 g (56%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.88 (m, 3H), 1.98 (p, J = 6.5 Hz, 2H), 2.53 (dt, J = 7.0, 1.5 Hz, 2H), 4.13 (t, J = 6.5 Hz, 2H), 5.52 (m, 1H), 6.03 (m, 1H), 9.75 (t, J = 1.5 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 18.3, 21.4, 40.6, 63.7, 125.7, 136.2, 167.3, 201.3.

***N*-Butylmethacrylateaminopyrenetrisulfonic acid trisodium salt (APTS-BuMA).** To a solution of APTS (0.6 g, 1.15 mmol) in dry methanol (20 mL) was added 2 (0.18 g, 1.15 mmol) and glacial acetic acid (1 mL, 17 mmol). A solution of sodium cyanoborohydride (0.3 g, 4.7 mmol) in dry methanol (10 mL) was then added, and the reaction was left stir at room temperature overnight. Starting material and product (~50:50) were observed by TLC, so the reaction was heated at 55 °C for 4 h. The reaction mixture was evaporated, and the resulting residue was re-dissolved in a minimal amount of water and purified by flash column chromatography on silica gel (isopropanol:NH_4OH, 9:1–3:1 gradient) to give 0.15 g (20% yield) of orange powder. TLC: fluorescent green spot, R_f = 0.5 in IPA/NH_4OH 2:1. Yield: 0.15 g (20%) UV–VIS (water) λ_{max} 457 nm (ε = 19,300 $M^{-1}cm^{-1}$). Fluorescence (pH 7.4 buffer) excitation maximum 463 nm, emission maximum 516 nm. ^{1}H NMR (CD_3OD, 500 MHz) δ 1.90 (m, 3H), 1.95 (m, 4H), 3.60 (t, J = 6.5 Hz, 2H), 4.26 (t, J = 6.0 Hz, 2H), 5.57 (m, 1H), 6.07 (m, 1H), 8.12 (s, 1H), 8.48 (d, J = 9.5 Hz, 1H), 8.88 (d, J = 9.5 Hz, 1H), 9.05 (d, J = 10 Hz, 1H), 9.13, (d, J = 9.5 Hz, 1H), 9.28 (s, 1H);^{13}C NMR (CD_3OD, 125 MHz) δ 16.98, 25.21, 26.09, 42.97, 64.34, 108.28, 116.67, 117.15, 120.44, 122.02, 123.55, 124.65, 124.72, 126.31, 126.64, 127.49, 130.19, 130.32, 134.52, 134.89, 136.33, 141.13, 144.05, 167.52; MS (ESI) m/z calcd for $C_{24}H_{24}NO_{11}S_3$ $(M + H)^+$: 598.04, found 598.1.

2-(Formylmethoxy)ethyl methacrylate (3). A solution of diethylene glycol monomethacrylate (4 g, 23 mmol) in dichloromethane (10 mL) was added to a suspension of pyridinium chlorochromate (7.4 g, 34.5 mmol) and celite (8 g) in dichloromethane (40 mL). The reaction was stirred at room temperature for 1 h. Diethyl ether (200 mL) was added and the reaction was filtered through celite. The dark brown filtrate was evaporated to a black oil which was then chromatographed on silica gel using 0% to 5% ethyl acetate in dichloromethane to yield 1.4 g (36% yield) of light green oil. Yield: 1.40 g (36%). ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.91 (s, 3H), 3.75 (m, 2H), 4.13 (m, 2H), 4.28 (m, 2H), 5.54 (s, 1H), 6.09 (s, 1H), 9.67 (s, 1H).

***N*-Ethoxyethyl methacrylate aminopyrenetrisulfonic acid trisodium salt (APTS-DEGMA).** To a solution of APTS (0.23 g, 0.44 mmol) in dry methanol (10 mL) was added 3 (0.3 g, 1.77 mmol) and glacial acetic acid (0.4 mL, 6.6 mmol). A solution of sodium cyanoborohydride (0.12 g, 1.77 mmol) in dry methanol (10 mL) was then added, and the reaction was left stir at 50 °C for 2 h. The reaction mixture was evaporated, and the resulting residue was re-dissolved in a minimal amount of methanol and purified by flash column chromatography on silica gel (isopropanol:NH_4OH, 7:1–4:1 gradient) to give 0.145 g (48% yield) of orange powder. TLC: fluorescent green spot, R_f = 0.3 in IPA/NH_4OH 3:1. Yield: 0.145 g (48%). UV–VIS (water) λ_{max} 454 nm (ε = 21,300 $M^{-1}cm^{-1}$). Fluorescence (pH 7.4 buffer) excitation maximum 462 nm, emission maximum 513 nm. ^{1}H NMR (CD_3OD, 500 MHz) δ 1.90 (m, 3H), 1.95 (m, 4H), 3.60 (t, J = 6.5 Hz, 2H), 4.26 (t, J = 6.0 Hz, 2H), 5.57 (m, 1H), 6.07 (m, 1H), 8.12 (s, 1H), 8.48 (d, J = 9.5 Hz, 1H), 8.88 (d, J = 9.5 Hz, 1H), 9.05 (d, J = 10 Hz, 1H), 9.13, (d, J = 9.5 Hz, 1H), 9.28 (s, 1H); ^{13}C NMR (CD_3OD, 125 MHz) δ 16.98, 43.10, 65.46, 68.73, 68.92, 108.39, 116.84, 117.53, 120.71, 121.77, 123.87, 124.69, 125.17, 126.26, 126.8, 127.45, 130.09, 130.24, 134.82, 135.16, 136.04, 141.11, 143.70, 167.44; MS (ESI) m/z calcd for $C_{24}H_{24}NO_{12}S_3$ $(M + H)^+$: 614.04, found 614.0.

Preparation of hydrogel containing APTS-BuMA (4). A 1 mL volumetric tube was charged with HEMA (353 mg, 2.7 mmol), PEGDMA (111 mg, 0.96 mmol), SPM (28 mg, 0.11 mmol), APTS-BuMA (0.2 mL of a 0.01 M solution in Nanopure water, 0.002 mmol), 2,2′-azobis[2-(2-imidazolin-2-yl)propane] dihydrochloride (VA-044) (2.4 mg, 7.4 μmol), and filled to the 1 mL mark with Nanopure water. The vial was sealed with a septum, agitated with a vortex mixer until a clear red solution was obtained, then deoxygenated by passing a stream of argon through the solution for about 10 minutes. The solution was then injected, by syringe, into a polymerization mold. The mixture was polymerized at 45 °C for 24 h.

Preparation of hydrogel containing APTS-DEGMA (5). A 1 mL volumetric tube was charged with HEMA (353 mg, 2.7 mmol), PEGDMA (111 mg, 0.96 mmol), SPM (28 mg, 0.11 mmol), APTS-DEGMA (0.2 mL of a 0.01 M solution in Nanopure water, 0.002 mmol), 2,2′-azobis[2-(2-imidazolin-2-yl)propane] dihydrochloride (VA-044) (2.4 mg, 7.4 μmol), and filled to the 1 mL mark with Nanopure water. The vial was sealed with a septum, agitated by a vortex mixer until a clear red solution was obtained, then deoxygenated by passing a stream of argon through the solution for about 10 minutes. The solution was then injected, by syringe, into a polymerization mold. The mixture was polymerized at 45 °C for 24 h.

Preparation of hydrogel containing HPTS-Lys-MA (6). A 1 mL volumetric tube was charged with HEMA (353 mg, 2.7 mmol), PEGDMA (111 mg, 0.96 mmol), SPM (28 mg, 0.114 mmol), HPTS-Lys-MA (0.2 mL of a 0.01 M solution in Nanopure water, 0.002 mmol), 2,2′-azobis[2-(2-imidazolin-2-yl)propane] dihydrochloride (VA-044) (2.4 mg, 7.4 μmol), and filled to the 1 mL mark with Nanopure water. The vial was sealed with a septum, agitated by a vortex mixer until a clear red solution was obtained, then deoxygenated by passing a stream of argon through the solution for about 10 minutes. The solution was then injected, by syringe, into a polymerization mold. The mixture was polymerized at 45 °C for 24 h.

Preparation of hydrogel containing 1MABP and APTS-BuMA (7). A 1 mL volumetric tube was charged with HEMA (353 mg, 2.7 mmol), PEGDMA (111 mg, 0.96 mmol), SPM (28 mg, 0.114 mmol), APTS-BuMA (0.2 mL of a 0.01 M solution in Nanopure water, 0.002 mmol), 1MABP (23 mg, 0.02 mmol), 2,2′-azobis[2-(2-imidazolin-2-yl)propane] dihydrochloride (VA-044) (2.4 mg, 7.4 μmol), and filled to the 1 mL mark with Nanopure water. The vial was sealed with a septum, agitated by a vortex mixer until a clear red solution was obtained, then deoxygenated by passing a stream of argon through the solution for about 10 minutes. The solution was then injected, by syringe, into a polymerization mold. The mixture was polymerized at 45 °C for 24 h.

Preparation of hydrogel containing 1MABP and APTS-DEGMA (8). A 1 mL volumetric tube was charged with HEMA (353 mg, 2.7 mmol), PEGDMA (111 mg, 0.96 mmol), SPM (28 mg, 0.114 mmol), APTS-DEGMA (0.2 mL of a 0.01 M solution in Nanopure water, 0.002 mmol), 1MABP (20.8 mg, 0.02 mmol), 2,2′-azobis[2-(2-imidazolin-2-yl)propane] dihydrochloride (VA-044) (2.4 mg, 7.4 μmol), and filled to the 1 mL mark with Nanopure water. The vial was sealed with a septum, agitated by a vortex mixer until a clear red solution was obtained, then deoxygenated by passing a stream of argon through the solution for about 10 minutes. The solution was then injected, by syringe, into a polymerization mold. The mixture was polymerized at 45 °C for 24 h.

Preparation of hydrogel containing 1MABP and APTS-DEGMA (9). A 1 mL volumetric tube was charged with HEMA (353 mg, 2.7 mmol), PEGDMA (111 mg, 0.96 mmol), SPM (28 mg, 0.114 mmol), APTS-DEGMA (0.1 mL of a 0.01 M solution in Nanopure water, 0.002 mmol), 1MABP (31.2 mg, 0.06 mmol), 2,2′-azobis[2-(2-imidazolin-2-yl)propane] dihydrochloride (VA-044) (2.4 mg, 7.4 μmol), and filled to the 1 mL mark with Nanopure water. The vial was sealed with a septum, agitated by a vortex mixer until a clear red solution was obtained, then deoxygenated by passing a stream of argon through the solution for about 10 minutes. The solution was then injected, by syringe, into a polymerization mold. The mixture was polymerized at 45 °C for 24 h.

Solution-based studies

pH Sensitivity studies. Fluorescence measurements were done *in situ* by taking the emission spectrum of APTS or HPTS over a pH range from 2.0–11.0. The pH was adjusted with either HCl or NaOH and checked with a Bruker 989 pH meter. The emission spectra of the dyes (4×10^{-6} M phosphate buffer) were initially measured at pH 7.4. The pH was then changed by addition of acid or base and the emission intensity was measured again at the resulting pH.

Stern–Volmer quenching studies. Fluorescence measurements were done *in situ* by taking the emission spectra of APTS or HPTS in pH 7.4 PBS containing a series of increasing quencher concentrations. The emission spectrum of the dye (APTS or HPTS, 2 mL of 4×10^{-6} M in buffer) was first obtained. The quencher (MABP) was then added (0.5–10 μL aliquots of a 5 mM solution in buffer), the solution was shaken for 30 sec, and a new emission spectrum was recorded after each quencher addition. Emission intensity was taken as the area under the curve from 480–650 nm for all studies. Stern–Volmer quenching constants were calculated using the sphere of action model,[23] eqn (1), where K_s represents the static quenching constant, V represents the dynamic quenching constant, and Q is the concentration of MABP. F_0 represents the initial fluorescence intensity of the dye only. F represents the fluorescence intensity at a given quencher concentration.

$$F_0/F = (1 + K_s[Q])e^{V[Q]} \quad (1)$$

Data was analyzed using Microcal Origin 7.5 from OriginLab, Northampton, MA (USA).

UV–VIS spectrophotometry of APTS and MABP. A 2.00 mL aliquot of APTS in phosphate buffer (pH 7.4, 39 mM) was added to a cuvette and the absorbance spectrum measured. To the cuvette were added successive aliquots of 0.5, 0.5, 1, 2, 4, 5, 5, and 10 μL MABP solution (20 mM in water). The total volume change was 1.5%. The solution was mixed by gentle shaking for 10 s after each addition. The spectrum was recorded after each titration.

Glucose response studies. Fluorescence measurements were done *in situ* by taking the emission spectrum of the quencher–dye solution at a series of increasing glucose concentrations. The emission spectrum of dye (APTS or HPTS, 2 mL of 4×10^{-6} M in buffer) was taken and quencher (MABP) was added (16 μL of 5 mM in buffer) to obtain a MABP/dye ratio of 10:1. After quencher addition, emission intensity was measured again. Glucose solution was then added (0.5–10 μL aliquots of 1 M in pH 7.4 buffer), the mixture was shaken for 30 sec, and the resultant intensity was measured after each addition. Fluorescence intensity was taken as the area under the curve between 480 nm and 650 nm for all studies. Apparent glucose binding constants were calculated by fitting the data with eqn 2.[24]

$$F/F_0 = (1 + (F_{max}/F_0)K_b[S])/(1 + K_b[S]) \quad (2)$$

where F_0 is the fluorescence intensity of the quenched dye, F is the fluorescence intensity after the addition of glucose, F_{max} is the intensity at which the fluorescence increase reaches its maximum, K_b is the apparent binding constant, and [S] is the concentration of glucose. Data was analyzed using Microcal Origin 7.5 from OriginLab, Northampton, MA (USA).

Hydrogel studies

Synthesis of hydrogels. All HEMA hydrogels were prepared in a similar manner except that the type and amount of dye and quencher were varied as noted in the text. The synthesis of hydrogel **7** is a representative example: a 1 mL volumetric tube was charged with HEMA (353 mg, 2.7 mmol), PEGDMA (111 mg, 0.96 mmol), SPM (28 mg, 0.114 mmol), dye (0.2 mL of a 0.01 M solution in Nanopure water, 0.002 mmol), 1MABP (23 mg, 0.02 mmol), 2,2′-azobis[2-(2-imidazolin-2-yl)propane] dihydrochloride (VA-044) (2.4 mg, 7.4 μmol), and filled to the 1 mL mark with Nanopure water. The vial was sealed with a septum, agitated on a vortex mixer until a clear red solution was obtained, then deoxygenated by passing a stream of argon through the solution for about 10 minutes. The solution was then injected, by syringe, into a polymerization mold, which was constructed in the following manner. Two 3 mm-thick glass plates (8 × 8 cm), one containing two small holes for solution injection, were treated with dichlorodimethylsilane (2% solution in toluene) to facilitate removal of the gel after polymerization. The treated plates were stacked atop each other separated by a 25.4 μm-thick Teflon spacer. The plates were clamped together with a metal frame that contained syringe injection ports aligned with the holes in the upper glass plate to form the mold. After the mold was filled with monomer solution and the holes closed with rubber septa, it was sealed in a Ziploc® bag that was purged and filled with argon, and heated in a 45 °C oven for 24 hours. The glass plates were then removed from the metal frame, placed in pH 7.4 phosphate buffer for 2 h, and separated to expose the thin orange/pink film. The film was cut into 1 × 3 cm pieces and immersed in phosphate buffer at 40 °C for 16 hours. They were then stored in fresh buffer solution at 5 °C until tested.

Fluorescence monitoring of hydrogel by front-face illumination. Measurements were performed using a Perkin-Elmer LS50-B luminescence spectrometer controlled by FL WinLab™ (version 2.0) software. The hydrogels were mounted in a flow-though cuvette, the cuvette was held in the spectrometer using a front surface accessory, and studies were carried out at 37 °C. A piece of hydrogel was mounted inside of the flow-through fluorescence cuvette in the following manner. While the cuvette (1 × 1 × 4 cm) was horizontal with both ends open, a piece of hydrogel was laid flat inside the cuvette. A stiff piece of 2 mm-thick black plastic (0.9 × 3 cm) with a window (0.7 × 2 cm) cut out of it was positioned on top of the gel. Using small forceps, two compression septa were placed on each end of the plastic frame, pressing it against the hydrogel. The polyethylene caps (appended with solvent inlet ports) were tightly sealed onto the cuvette using vacuum grease and Teflon tape. The cuvette was held in the spectrometer with the front surface accessory and oriented so that solutions entered through the bottom and flowed upward. The side of the cuvette against which the gel was pressed, was facing the excitation source at a 30° angle relative to the incident beam. Phosphate buffer (37 °C) was pumped into the cell at a flow rate of 12 mL/min. The film was excited at 470 nm by front-face illumination, and the emission was monitored over time. A 515 nm cutoff filter was used, and the excitation and emission slit widths were adjusted between 7 and 10 nm to obtain a baseline intensity of ~400 intensity units (1000 unit limit). Using the time drive application, the integration time was set for 10 sec, and the source was pulsed every 2 sec. After a stable signal was obtained (~1 h), buffered glucose solutions of varying concentrations were pumped through the cuvette.

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References

1 (*a*) L. Heinemann and G. Schmelzeisen-Redeker, *Diabetologia*, 1998, **41**, 848–854; (*b*) R. J. McNichols and G. L. Cote, *J. Biomed. Opt.*, 2000, **5**, 5–16; (*c*) O. S. Khalil, *Clin. Chem.*, 1999, 165; (*d*) T. Koschinsky and L. Heinemann, L., *Diabetes/Metab. Res. Rev.*, 2001, **17**, 113–123.
2 (*a*) G. Van den Berghe, P. Wouters, F. Weekers, C. Verwaest, F. Bruyninckz, M. Schetz, D. Vlasselaers, P. Ferdinande, P. Lauwers and R. Bouillon, *N. Engl. J. Med.*, 2001, **345**, 1359–1367; (*b*) G. Van den Berghe, *Int. J. Obes.*, 2002, **26**, S3–S8; (*c*) S. J. Finney, C. Zekland, A. Elia and T. W. Evans, *J. Am. Med. Assoc.*, 2003, **290**, 2041–2047.
3 For reviews see: (*a*) J. Wang, *Sens. Update*, 2002, **10**, 107–119; (*b*) G. S. Wilson and Y. Hu, Y., *Chem. Rev.*, 2000, **100**, 2693–2704; (*c*) P. U. Abel and T. von Woedtke, *Biosens. Bioelectron.*, 2002, **17**, 1059–1070; (*d*) M. Staiano, P. Bazzicalupo, M. Rossi and S. D'Aurio, *Mol. BioSyst.*, 2005, **1**, 354–362.
4 E. A. Moschou, B. V. Sharma, S. K. Deo and S. Daunert, *J. Fluoresc.*, 2004, **14**, 535–547.
5 (*a*) T. D. James and S. Shinkai, *Top. Curr. Chem.*, 2002, **218**, 159–200; (*b*) H. S. Mader and O. S. Wolfbeis, *Microchim. Acta*, 2008, **162**, 1–24.
6 See for example: US Pat., 6 002 954, 1999; US Pat., 6 304 766, 2001; US Pat., 6 387 672, 2002; US Pat., 6 627 177, 2003; US Pat., 7 297 548, 2007.
7 (*a*) J. Yoon and A. W. Czarnik, *J. Am. Chem. Soc.*, 1992, **114**, 5874–5875; (*b*) S. Arimori, C. J. Ward and T. D. James, *J. Chem. Soc., Chem. Commun.*, 2001, 2018–2019; (*c*) H. Eggert, J. Frederiksen, C. Morin and J. C. Norrild, *J. Org. Chem.*, 1999, **64**, 3846–3852; (*d*) W. Yang, H. He and D. G. Drueckhammer, *Angew. Chem., Int. Ed.*, 2001, **40**, 1714–1718; (*e*) A. J. Tong, A. Yamauchi, T. Hayashita, Z. Y. Zhang, D. B. Smith and N. Teramae, *Anal. Chem.*, 2001, **73**, 1530–1536; (*f*) D. P. Adhikiri and M. D. Heagy, *Tetrahedron Lett.*, 1999, **40**, 7893–7896; (*g*) N. DiCesare and J. R. Lakowicz, *Tetrahedron Lett.*, 2001, **42**, 9105–9108.
8 (*a*) S. Arimori, C. J. Ward and T. D. James, *Tetrahedron Lett.*, 2002, **43**, 303–305; (*b*) G. Springsteen and B. Wang, *J. Chem. Soc., Chem. Commun.*, 2001, **17**, 1608–1609; (*c*) G. Springsteen and B. Wang, *Tetrahedron*, 2002, **58**, 5291–5300; (*d*) S. L. Wilkur, H. Ait-Haddou, J. J. Lavigne and E. V. Anslyn, *Acc. Chem. Res.*, 2001, **34**, 963–972; (*e*) S. Arimori, H. Murakami, M. Takeuchi and S. Shinkai, *J. Chem. Soc., Chem. Commun.*, 1995, **9**, 961–962; (*f*) N. DiCesare, M. R. Pinto, K. S. Schanze and J. R. Lakowicz, *Langmuir*, 2002, **18**, 7785–7787.
9 (*a*) T. D. James, K. Sandanayake and S. Shinkai, *Angew. Chem., Int. Ed. Eng.*, 1994, **33**, 2207–2209; (*b*) T. D. James, K. Sandanayake and S. Shinkai, *J. Chem. Soc., Chem. Commun.*, 1994, 477–478; (*c*) T. D. James, K. Sandanayake, R. Iguchi and S. Shinkai, *J. Am. Chem. Soc.*, 1995, **117**, 8982–8987; (*d*) K. Sandanayake, S. Imazu, T. D. James, M. Mikami and S. Shinkai, *Chem. Lett.*, 1995, 139–140; (*e*) T. D. James, K. Sandanayake and S. Shinkai, *Angew. Chem., Int. Ed. Eng.*, 1996, **35**, 1911–1922; (*f*) T. D. James, H. Shinmori and S. Shinkai, *Chem. Commun.*, 1997, 71–72.
10 J. P. Lorand and J. O. Edwards, *J. Org. Chem.*, 1959, **24**, 769–774.
11 (*a*) J. N. Camara, J. T. Suri, F. E. Cappuccio, R. A. Wessling and B. Singaram, *Tetrahedron Lett.*, 2002, **43**, 1139–1141; (*b*) J. T. Suri, D. B. Cordes, F. E. Cappuccio, R. A. Wessling and B. Singaram, *Langmuir*, 2003, **19**, 5145–5152; (*c*) J. T. Suri, D. B. Cordes, F. E. Cappuccio, R. A. Wessling and B. Singaram, *Angew. Chem., Int. Ed. Engl.*, 2003, **42**, 5857–5859; (*d*) F. E. Cappuccio, J. T. Suri, D. B. Cordes, R. A. Wessling and B. Singaram, *J. Fluoresc.*, 2004, **14**, 521–533.
12 S. Gamsey, J. T. Suri, R. A. Wessling and B. Singaram, *Langmuir*, 2006, **22**, 9067–9074.
13 C. A. Burtis and Tietz, in *Textbook of Clinical Chemistry*, 3rd edn, WB Saunders Co., Pennsylvania, 1998.
14 R. A. Evangelista, A. Guttman and F.-T. A. Chen, *Electrophoresis*, 1996, **17**, 347–351.
15 D. B. Cordes, A. Miller, S. Gamsey, Z. Sharrett, P. Thoniyot, R. A. Wessling and B. Singaram, *Org. Biomol. Chem.*, 2005, **3**, 1708–1713.
16 APTS can be purchased from Fluka as a fluorescent labeling reagent for glycoproteins and saccharides for $158/10 mg. 1-Aminopyrene can be purchased for $18/250 mg.
17 (*a*) D. B. Cordes, S. Gamsey, Z. Sharrett, A. Miller, P. Thoniyot, R. A. Wessling and B. Singaram, *Langmuir*, 2005, **21**, 6540–6547; (*b*) S. Gamsey, A. Miller, M. M. Olmstead, C. M. Beavers, L. C. Hirayama, S. Pradhan, R. A. Wessling and B. Singaram, *J. Am. Chem. Soc*, 2007, **129**, 1278–1286.
18 (*a*) O. S. Wolfbeis and Z. Fresenius, *Anal. Chem.*, 1985, **320**, 271–273; (*b*) S. G. Schulman, S. Chen, F. Bai, M. J. P. Leiner, L. Weis and O. S. Wolfbeis, *Anal. Chim. Acta*, 1995, **304**, 165–170; (*c*) K. A. Giuliano and R. J. Gillies, *Anal. Biochem*, 1987, **167**(2), 362–371.
19 (*a*) S. Gamsey, A. Miller, M. M. Olmstead, C. M. Beavers, L. C. Hirayama, S. Pradhan, R. A. Wessling and B. Singaram, *Tetrahedron*, 2006, **62**, 6321–6331; (*b*) Z. Sharrett, S. Gamsey, J. Fat, D. Cunningham-Bryant, R. A. Wessling and B. Singaram, *Tetrahedron Lett.*, 2007, **48**, 5125–5129.
20 (*a*) K. Park, W. S. W. Shalaby and H. Park, in *Biodegradable Hydrogels for Drug Delivery*, Technomic Publishing, Lancaster, PA, 1993; (*b*) S. W. Kim, Y. H. Bae and T. Okano, *Pharmacol. Res.*, 1992, **9**, 283–290.
21 C. Wang and W. Cao, *Polym. Int.*, 1996, **41**, 449–451.
22 (*a*) A. Schiller, R. A. Wessling and B. Singaram, *Angew. Chem., Int. Ed.*, 2007, **46**, 6457–6459; (*b*) A. Schiller, B. Vilozny, R. A. Wessling and B. Singaram, *Anal. Chim. Acta*, 2008, **627**(2), 203–211.
23 (*a*) J. R. Lakowicz, in *Principles of Fluorescence Spectroscopy*, 3rd edn., Springer, 2006, ch. 8, pp. 278–286; (*b*) D. L. Wang, J. Wang, D. Moses, G. C. Bazan and A. J. Heeger, *Langmuir*, 2001, **17**, 1262–1266.
24 K. A. Connors, in *Binding Constants- The Measurement of Molecular Complex Stability* Wiley-Interscience, 1987.

