

# Fluorescent acrylamide nanoparticles for boronic acid based sugar sensing — from probes to sensors

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**Abstract** Fluorescent nanoparticles containing covalently bound phenylboronic acids (~ 250 nm in diameter) are presented that respond to carbohydrates by swelling which is detected using fluorescence resonance energy transfer. The nanoparticles are characterized in terms of kinetics, response time and dynamic range. The response of the particles to glucose at pH 7.5 depends on the kind of phenylboronic acid and on ionic strength. The particles were immobilized in hydrogel sensor layers that enable continuous optical sensing of carbohydrates.

**Keywords** Stimuli responsive polymers · Nanoparticles · Fluorescence · Glucose sensors · Phenylboronic acid · Swelling

## Introduction

Smart polymers, which change one of their properties upon an external stimulus, have been of great interest for sensor technology in the past years [1]. Typical stimuli include physical events like change in heat [2] or light [3] and the presence of chemical analytes like pH [4, 5], and other molecules [6]. Among these, glucose represents one of the most interesting analytes, not only for biochemical appli-

cations like cellular assays but also in the medical field, where especially continuous glucose measurement is still a non trivial task [7].

Glucose measurement with hydrogels has gained much interest according to recent literature [8]. Various approaches have been investigated, utilizing different glucose receptors and detection methods. The enzyme *glucose oxidase* was immobilized in a hydrogel and the change of pH in the system—triggered by the oxidation of the glucose to gluconic acid—forced the hydrogel to swell. This swelling can also be used to set previously entrapped insulin free. The hydrogel therefore cannot only sense glucose, but also act as a drug delivery system as well [6]. The swelling of these systems can be read out with mass-sensitive magnetoelastic sensors [9] or a microscope [10]. But the use of an enzyme as receptor also bears some disadvantages like limited pH and temperature range, and activity loss when immobilized and over time.

These limitations can be overcome by using a synthetic glucose receptor. Phenylboronic acids are known to reversibly form an ester with 1,2- and 1,3-cis diols in aqueous solutions, and have been used for sugar sensing for some time [11]. Since the ester is much more stable when the phenylboronic acid is in its tetrahedral state, the phenylboronic acid becomes negatively charged upon sugar binding. When the phenylboronic acid is immobilized in a hydrogel, the addition of glucose, or other 1,2- or 1,3 diols, induces a negative charge in the hydrogel by reacting with the phenylboronic acid. Depending on the surrounding environment, an osmotic pressure is generated due to the resulting Donnan potential [12]. This causes water to stream into the hydrogel and therefore enlarge its volume. The swelling process is also induced and amplified by the charge repulsion of the negatively charged phenylboronic acids. Additionally a

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volume transition is induced via the change of affinity for water, since the phenylboronic acid becomes more hydrophilic when it is complexed with sugar. This process enables for the measurement of sugars at high ionic strength because this induced change of the lower critical solution temperature is still valid at high ionic strength. The properties of such hydrogels have been investigated and reported in great detail [13–20].

An interesting approach is the introduction of an amine group in the system [21]. This causes the hydrogel to be swollen in the initial state and the addition of glucose can be selectively be measured: Only glucose can form a 2:1 phenylboronic acid:sugar complex which causes the hydrogel to crosslink and shrink in size [22]. This system was used in the first real application of a sensor based on phenylboronic acids used to measure glucose in human blood plasma [23, 24]. Other approaches utilize the change of the optical density [25, 26] or use polymer colloidal crystals [27–30] for the determination of glucose concentrations.

Recently we published a study on fluorescent sugar responsive nanospheres based on the swelling of nanoparticles [31]. These nanospheres were composed of slightly crosslinked poly(*N*-isopropylacrylamide) (NIPAM) with 3-acrylamidophenylboronic acid (ACPBA) as sugar receptor. Additionally, two fluorophores which have overlapping emission and excitation spectra were copolymerized into the nanoparticles. Based on the distant dependent resonance energy transfer, we were able to track the sugar induced swelling of nanoparticles by the change in the fluorescence emission spectra of the particles. Without sugar present, the fluorescent donor and acceptor are in close proximity and the fluorescence of the donor is quenched by the acceptor. After addition of sugar, the particles begin to swell due to the above mentioned processes. The distance between the fluorescent donor and acceptor is increased, leading to a dramatic change in the fluorescence emission spectra. With this technique we could monitor the response of the nanoparticles to various glucose / fructose concentrations. The

carbohydrate concentrations needed to trigger a swelling were rather high (100 mM glucose at pH 8.5) and the swelling took about 100 min to reach equilibrium size.

In this article we want to report our progress in enhancing this system to be able to swell faster at lower sugar concentrations. We also investigate the influence of pH, and ionic strength on the measurements, and want to demonstrate the reversibility of the swelling process by immobilizing these particles in a hydrogel and show the response to inclining and declining sugar concentrations.

## Experimental

Materials, synthesis of the monomers and measurement methods can be found in the [Supporting Information](#).

### Synthesis of the polymer nanospheres

Stock solutions of Sodiumdodecylsulfate (SDS) (5 mg mL<sup>-1</sup>), N,N'-methylenebis(acrylamide) (BIS) (10 mg mL<sup>-1</sup>), N-fluorescein acrylamide (FLAC) (0.08 mg mL<sup>-1</sup>) and acryloylpiperazinyl sulforhodamine B (ASRB) (0.15 mg mL<sup>-1</sup>) were made. The solid monomers and liquid solutions were mixed according to Table 1 and filled up with water to 10 mL. This solution was held at 69 °C while bubbling with N<sub>2</sub> to ensure the exclusion of oxygen. After at least 30 min, ammonium peroxodisulfate (APS) (dissolved in 1 mL water) was added to the mixture under vigorous magnetic stirring. Upon successful initiation of the polymerization, the solution became turbid after 10 min. Nitrogen was left bubbling for 45 min. and the mixture was then allowed to stir at 69 °C for another 45 min.

After the suspension had cooled, it was transferred into a dialysis membrane and dialyzed against water for at least 4 days with exchange of water twice a day. The aqueous solution was lyophilized to yield a pink foam. (Yields are listed in Table 2).

**Table 1** Monomer feed composition of the different polymers

| Name | NIPAM<br>mg (wt%) | BIS <sup>a</sup><br>mg (wt%) | Boronic acid<br>mg (wt%) | FLAC <sup>a</sup><br>mg (wt%) | ARSB <sup>a</sup><br>mg (wt%) | SDS <sup>a</sup><br>mg | APS<br>mg |
|------|-------------------|------------------------------|--------------------------|-------------------------------|-------------------------------|------------------------|-----------|
| AM1  | 141 (94.51)       | 1.6 (1.07)                   | 6.1 (4.09) <sup>b</sup>  | 0.41 (0.28)                   | 0.08 (0.05)                   | 2.5                    | 10        |
| AM2  | 212 (95.79)       | 3.0 (1.01)                   | 5.8 (2.62) <sup>b</sup>  | 0.38 (0.17)                   | 0.15 (0.07)                   | 2.5                    | 10        |
| AL1  | 120 (89.59)       | 1.75 (1.31)                  | 12 (5.18) <sup>c</sup>   | 0.08 (0.06)                   | 0.13 (0.09)                   | 2.5                    | 6         |

<sup>a</sup> Added via stock solutions, see text

<sup>b</sup> 3-Acylamidophenylboronic acid

<sup>c</sup> 4-Allylaminocarbonylphenylboronic acid

**Table 2** Properties of the nanoparticles

| Name | Yield (%) | Boronic acid in feed (wt.%) | Boronic acid in polymer (wt.%) <sup>a</sup> | Size (nm)        | PDI  |
|------|-----------|-----------------------------|---|------------------|------|
| AM1  | 90        | 4.09                        | 4.78  | 232 <sup>b</sup> | 0.04 |
| AM2  | 90        | 2.62                        | 1.87  | 294 <sup>b</sup> | 0.02 |
| AL1  | 35        | 5.18                        | 4.97  | 162 <sup>c</sup> | 0.16 |

<sup>a</sup> determined knowing the dried mass using ICP-OES

<sup>b</sup> at pH 8.5; ionic strength 154 mM; 25 °C

<sup>c</sup> at pH 8.0; ionic strength 154 mM; 25 °C

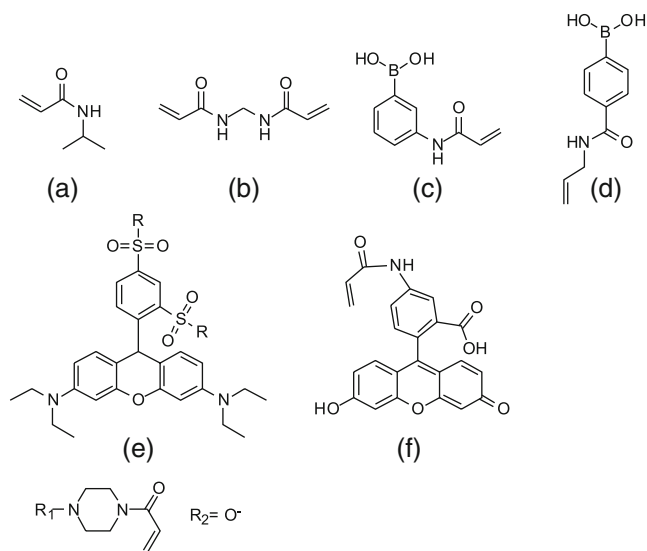
### Sensor film preparation

The particles were dispersed in ethanol (8.5 mg.mL<sup>-1</sup>) and mixed 1:1 with a 5 wt.-% solution of Hydromed D6 in ethanol/water 9/1. After thorough vortexing, this cocktail was spread on the dust free PET support by a homemade knife coating device to obtain thin films of a calculated thickness of approximately 30 μm. The films were left in the fume hood for one night to ensure complete evaporation of the solvents.

### Results and discussion

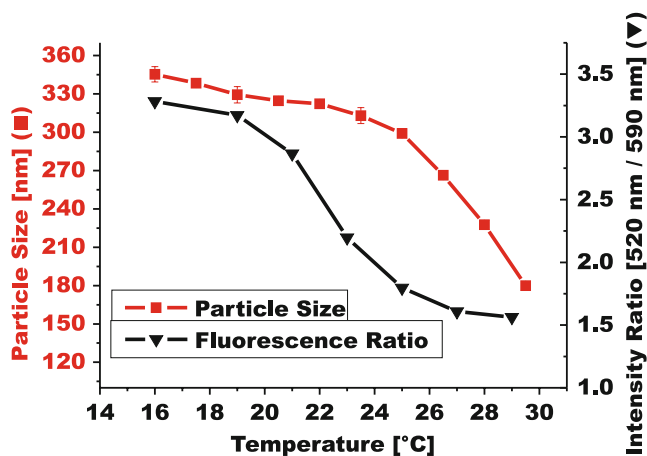
#### Synthesis and characterization of the particles

The stimuli responsive nanospheres were prepared via a radical precipitation polymerization of all monomers (see Fig. 1). After dialysis to remove all unreacted monomers and lyophilization of the suspension, the particles could be



**Fig. 1** Structures of the monomers used for the particle synthesis: **a** N-isopropylacrylamide (NIPAM); **b** N,N'-methylenebis(acrylamide) (BIS); **c** 3-acrylamidophenylboronic acid (ACPBA); **d** 4-allylaminocarbonylphenylboronic acid (AAPBA); **e** N-fluorescein acrylamide (FLAC); **f** acryloylpiperazine sulforhodamine B (ARSB)

redispersed by ultrasonication for 10 min. Analysis by ICP-OES revealed that the phenylboronic acid content in the polymer is almost the same as in the monomer feed, which is in accordance to similar experiments [13, 32]. Compared to our previous report, more SDS was used in the synthesis which resulted in the formation of smaller particles (see Table 2 and Fig. 2). Depending on pH and temperature of the measurement conditions the particles have a size of ~250 nm. In Fig. 2 we show the correlation of the particle size and the fluorescence intensity ratio as a function of temperature. Particles made from NIPAM are known to change their size when the temperature is changed, due to the thermoresponsive properties of NIPAM [32]. We use this phenomenon to show that we can track the change of the particle size via the fluorescence intensity ratio. With changing temperature, the size, and as a result of this, the emission intensities of the particle change. The dynamic range of the fluorescent size measurement is dependent on the size of the particle: When the particle is in its smallest state (at 29 °C), almost all of the donor molecules are thought to be in close



**Fig. 2** Correlation of the temperature, the particle size and the fluorescence of the particles. As the temperature is increased, the particles begin to shrink, due to the thermoresponsive properties of NIPAM. A change of the fluorescence intensity can also be observed which shows that the particle size and the fluorescent spectra are correlated. At temperatures larger than 31 °C the particles aggregate. (Particles AM1; pH 9.0, ionic strength 154 mM,  $\lambda_{ex}$ : 470 nm)

proximity to the acceptor molecules. As the particle gains in size, the probability of an energy transfer is reduced, as more and more of the donor molecules have no acceptor in close proximity. The particles show a critical flocculation temperature at temperatures higher than 30–31 °C [33]. The terms “swelling” and “equilibrium sizes” used in this work are based on the observed fluorescence intensity ratio change. Neither the extent of the change in size nor the particle size itself can be determined with these measurements, but as we intend to use these particles as analytical tools rather than investigating the structural changes, these absolute values are of little interest.

The influence of the pH on the particle size of particles AM1 (see Table 1) at various temperatures can be seen in Fig. S1 in the [Supporting Information](#). At pH higher than 8.0 the phenylboronic acids become ionized which results in a more swollen state at a certain temperature than at a pH lower than 8.0

The fluorescence emission spectrum of the particles in the shrunken and swollen state, induced either by temperature or sugar, can be seen in Fig. S2 in the [Supporting Information](#). As the particle swells, the emission intensity of the donor increases, as less acceptor is available for quenching. This can be also seen in the fluorescence spectrum as the acceptor peak is only visible as a shoulder in the emission spectrum at the swollen state.

The low polydispersity index of the particles measured by dynamic light scattering is also represented in the electron microscopy picture in Fig. S3 in the [Supporting Information](#). Since the particles are dry, the size which is measured under the microscope is lower than in solution, but the homogenous size distribution and the round and uniform shape is evident. This shows that these particles can be used as probes and later on as sensors for carbohydrates.

#### Response of the particles with 3-acrylamidophenylboronic acid to glucose and fructose

As we have previously shown, the response of the particles to sugar in the form of swelling can be tracked via fluorescence conveniently in a microplate, which allows for multiple measurements in parallel. For the experiments we use glucose and fructose as analyte molecules, as glucose is an attractive analyte molecule due to its important role in metabolism, and fructose is known to have a very high association constant with phenylboronic acid [34]. The phenylboronic acid used in the nanoparticles has a  $pK_a$  of 8.2 in solution but in the polymer matrix the  $pK_a$  is increased to 8.9 [35].

The response of the particles AM1 (see Table 1) with 4.8 wt.-% ACPBA to different glucose concentrations at pH 8.5 and 9.0 is shown in Fig. 3. After the addition of sugar, the

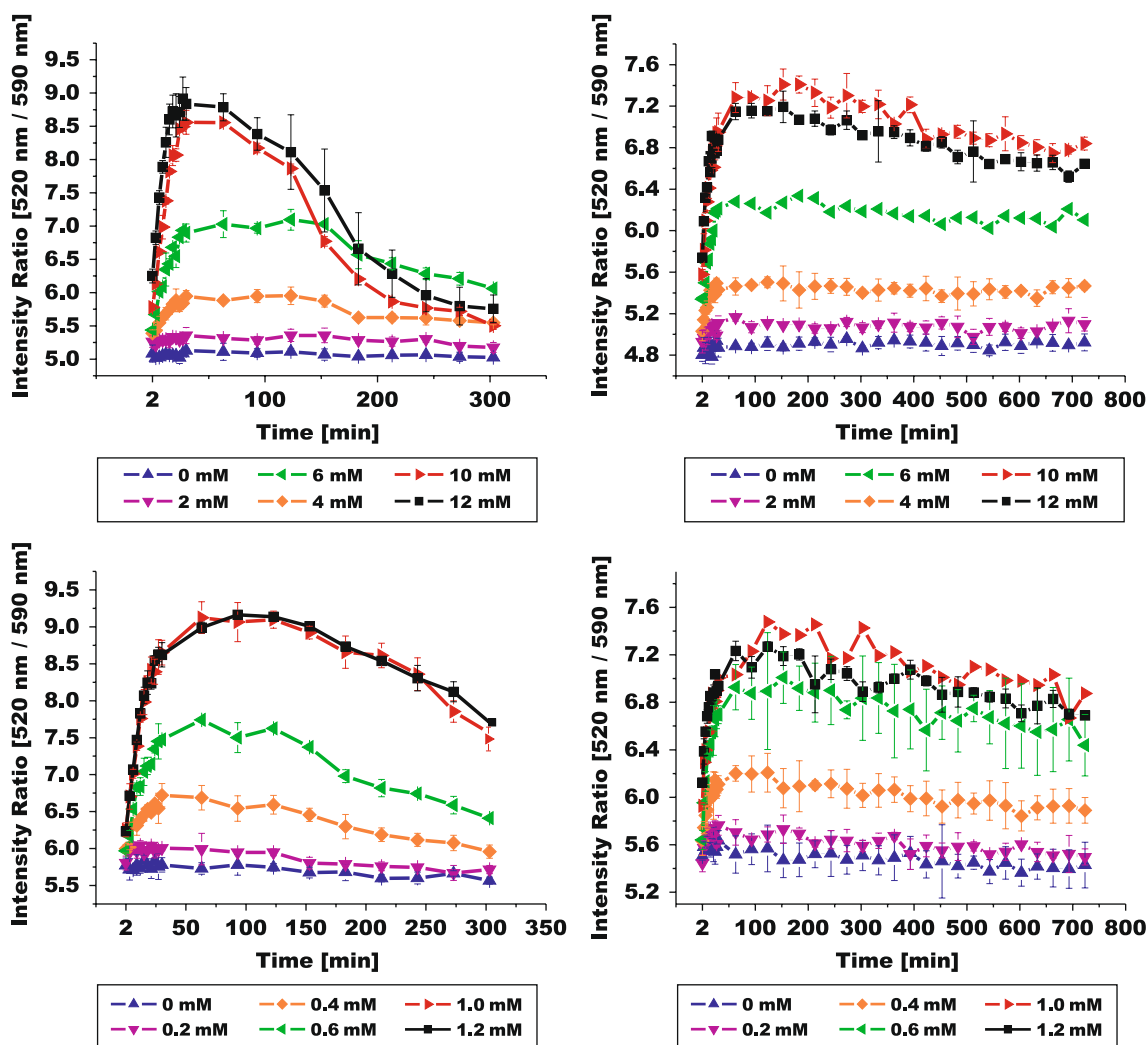
particles swell to certain degree, depending on the kind and concentration of the sugar and the pH at which the measurement was done. By lowering the phenylboronic acid concentration in the particle, the time, after which an equilibrium size is reached, is reduced to 50 min compared to 200 min with the particles with 20 wt.-% which we presented in our previous publication.

The influence of the phenylboronic acid content on the response of polymers to sugars has been investigated by several groups [36, 37]. Lee et al. have found that for their holographic sensors 20 mol.-% of ACPBA resulted in the highest sensitivity. Kuzimenkova et al. have shown that for the determination of glucose via optical density changes in polymer gels an ACPBA content of 9.1 mol.-% is beneficial and that the increase to 10.2 mol.-% resulted in slower and weaker response to glucose. Both applications used bulk hydrogels rather than polymers in the nanoscale as used in this work. Apparently by reducing the size of the sensing units a reduction of the phenylboronic acid content to lower than 5 wt.-% is improving the response of the probes, because the small charged fraction is able to trigger the swelling process in a small particle.

Different carbohydrate concentrations lead to different fluorescence intensity ratios, which render the particles useful as sensor materials. The influence of the pH value on the reaction of the particles with glucose can be seen by comparing the upper (pH 8.5) and the lower half (pH 9.0) of Fig. 3. At pH 9.0, where the pH roughly equals the  $pK_a$  of the phenylboronic acid, only a tenth of the glucose concentrations were needed to elicit a reaction with glucose compared to the measurements made at pH 8.5.

The influence of the ionic strength on the measurement can be seen by comparing the left (154 mM ionic strength) and right (50 mM ionic strength) side of Fig. 3. At high ionic strength a shrinking of the particles can be observed (left part). During our research on this unexpected phenomenon, we were able to rule out one possible explanation, the internal crosslinking of the particles due to the formation of a 2:1 phenylboronic acid:glucose complex [38]: The reaction of the particles with catechol, which has a high association constant with phenylboronic acid, at the same conditions also showed this shrinkage of the particles. Due to the chemical structure of catechol, no crosslinking can occur with this molecule.

Currently we do not know the reason behind this shrinking. The ionic strength is able to change the extent of the swelling, so this might be a parameter which is responsible for it. In the measurements at lower pH, which will be presented in the next section, hardly any shrinking was observed (During the review process one of the reviewers pointed out that maybe an increased penetration of salts into the swollen particle or decreased electrostatic



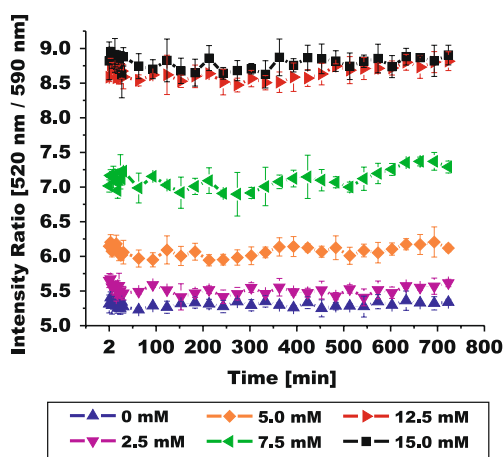
**Fig. 3** Response of the particles AM1 to glucose at pH 8.5 at 154 mM (*top left*) and 50 mM (*top right*) ionic strength and to glucose at pH 9.0 at 154 mM (*bottom left*) and 50 mM (*bottom right*) ionic strength

repulsion is the reason behind this phenomenon). As we intend to use these particles as a glucose sensor at physiological conditions, the shrinking should not affect this application.

Measurements at pH values below the  $pK_a$  of the phenylboronic acid were also possible, albeit higher sugar concentrations were needed to trigger a response compared to measurements at higher pH. The pH influences the reaction of phenylboronic acid bearing hydrogels via the degree of ionized phenylboronic acid groups that are available for sugar binding [21]. The closer the pH of the reaction medium is to the  $pK_a$  of the phenylboronic acid, the more ionic phenylboronic acid groups are available and therefore the sugar concentrations needed for the triggering of the swelling are lower. Furthermore the particles are slightly swollen at higher pH values which eases the diffusion of sugar into the particle and makes more phenylboronic acids available for sugar binding. (See Fig. S1 in the [Supporting Information](#)).

Measurements at pH values below the  $pK_a$  of the phenylboronic acid (pH 7.5–8) were also possible, albeit higher sugar concentrations were needed to trigger a response compared to measurements at higher pH. For example compared to the measurements at pH 9.0, a 10-fold increase in glucose concentration was needed to achieve a response and the same dynamic range at pH 8.5 and a roughly an 80-fold increase was needed for the pH 8.0 glucose concentration. In Fig. 4, one can see that particles AM2 are already at their equilibrium sizes at pH 7.5 with fructose, right after the addition of the sugar, before the first measurement cycle. Detailed examination of the samples has revealed that the equilibrium size has been reached after 1.5 min of the actual sugar addition (see Figure top left part of Fig. S4 in the [Supporting Information](#)), which makes the particles swell faster than at higher pH values. This can be attributed to the fact that phenylboronic acids are in neutral state at pH 8.0 and 7.5 due to their high  $pK_a$  and therefore the reaction with sugar





**Fig. 4** Response of particles AM2 to fructose at pH 7.5 (ionic strength 154 mM). For a more detailed investigation of the fast response times, the response to glucose and the calibration curves see Fig. S4 in the [Supporting Information](#)

induces the formation of negative charged phenylboronic acids, while at higher pH the phenylboronic acids are thought to be initially (at least partially) negatively charged and the sugar addition just shifts the equilibrium in the direction towards more negative charged phenylboronic acids. This fast response time is the result of the small sensor unit (in the nanometer scale) and the very strong association of fructose to ACPBA even at pH 7.5. In contrast, the reaction of the particles with glucose takes about 10 min to reach an equilibrium swelling at pH 8.0 (lower part of Fig. S4 in the [Supporting Information](#)). No reaction of the particles with glucose was observed at pH 7.5 even at a level of 400 mM glucose. It seems that the low association constant of glucose and this phenylboronic acid combined with the low pH prevents the particles from swelling. The particles AM1 respond similar to the sugars at this pH, but the average response times are 12 min for fructose and 25 min for glucose. Since the difference between these particles only lies in the amount of phenylboronic acid (AM1: 4.09 wt.-% AM2: 2.62 wt.-%) the amount of phenylboronic acid seems to be crucial for short response times. This can also be seen in the reduced response times compared to the particles with 20 wt.-% phenylboronic acid content noted earlier in this article.

While the pH and the  $pK_a$  are the dominating factors behind the different swelling kinetics at the various pH values, it should also be noted that the particles are smaller at lower pH values. This makes the diffusion of the sugar into the particles much more difficult and the phenylboronic acids are less accessible at smaller sizes.

All particles show a constant fluorescence intensity ratio for over 12 h. This, and almost linear calibration curves shows a potential application of this measurement setup in analytical chemistry.

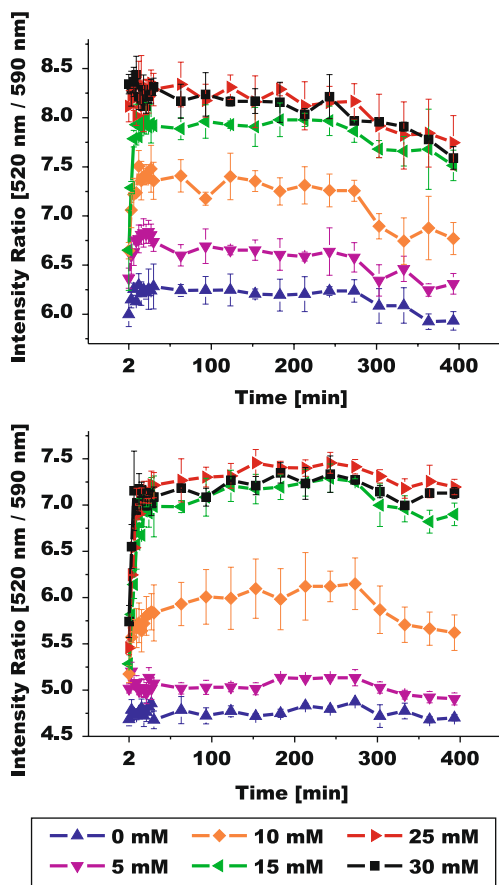
Particles containing a phenylboronic acid with lower  $pK_a$

The  $pK_a$  of the phenylboronic acid determines the pH and the concentration range at which a sugar forms the complex with the acid. A  $pK_a$  of the phenylboronic acid close to 7.4 is beneficial for measurements at the physiological pH which is important for measurements in real life samples. The  $pK_a$  of the phenylboronic acid is greatly influenced by the position of the substituents on the phenyl ring. AAPBA is one of the few commercially available phenylboronic acid with a monomeric unit not in meta position to the phenylboronic acid group. Via potentiometric titration, we found the  $pK_a$  of AAPBA to be  $7.93 \pm 0.07$  in solution which is in good agreement with a similar synthesized structure [35] and significantly lower than the  $pK_a$  of the previous used ACPBA ( $pK_a$  of 8.2 in solution [35]) (See [Supporting Information](#) Fig. S5 for titration curves and a plot of the ionization degree vs. pH).

Sugar responsive fluorescent particles with this receptor (AL1) can be prepared in the same manner as with the particles using ACPBA as receptor. The yield of the particles is lower compared to the particles with ACPBA and the resulting particles are smaller in diameter (see Table 2). The fluorescence emission spectra of the particles with and without glucose at pH 7.5 can be seen in Fig. S2 in the [Supporting Information](#).

The response of these particles to glucose at pH 8 with different levels of ionic strength is shown in Fig. 5. Compared to the particles AM1, which have comparable phenylboronic acid content, particles AL1 swell at significantly lower glucose concentrations. The concentration needed to trigger a response at pH 8.0—a pH close to the  $pK_a$  of this phenylboronic acid—is lowered by 66% and the particles show a stable equilibrium swelling degree for at least 200 min. The ionic strength influences the dynamic range and the response time of the particles. At 5 mM ionic strength (top part of Fig. 5) the dynamic range lies between 0 and 25 mM glucose while at 200 mM ionic strength (lower part of Fig. 5) the dynamic range is lowered to 0 and 15 mM. The response time of the particles is slightly slower at higher ionic strengths. While the particles at 5 mM are at their equilibrium size after 6 min, it takes them 12 min to reach that size at 200 mM. At 0 mM glucose concentration, the intensity ratios are different for 5 mM and 200 mM ionic strength which represents different sizes of the particles at the respective ionic strength. The initially slightly bigger size of the particles at 5 mM ionic strength could be the reason for the faster swelling [13].

The influence of the ionic strength on the response can also be seen in the measurements of glucose at pH 7.5, where high ionic strength prevent the particles from swelling at all (see Fig. S7 [Supporting Information](#)). At an ionic strength of 50 mM, a response to glucose can be measured, which was



**Fig. 5** Kinetic responses of the particles AL1 to glucose at pH 8.0. *Top*: Ionic strength 5 mM (Buffer concentration 5 mM); *Bottom*: Ionic strength 200 mM (Buffer concentration 50 mM). Compared to the particles AM2 (see Fig. S4 in the Supporting Information) lower glucose concentrations are needed to trigger a response. For the calibration curves of these measurements see Fig. S6 in the Supporting Information

not possible with the particles AM1/2. The higher the ionic strength is in the system, the smaller the particles get initially without glucose (see the 0 mM concentration in Fig. S7 Supporting Information). Correlating with this, at high levels of ionic strength small or no change of the fluorescence intensity can be observed.

At pH values higher than 8.0, the particles are initially swollen to an extent, which renders them unusable for sugar measurement, as no change in the fluorescence after addition of sugar was observed. This is due to the high fraction of already negatively charged phenylboronic acid groups due to the low  $pK_a$  of the phenylboronic acid.

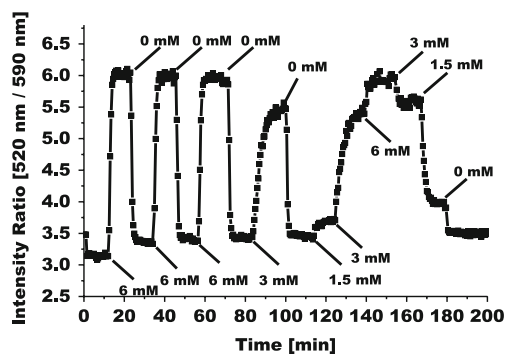
Immobilization of the particles—from measurements in solution to application

To make the transition from a sugar responsive probe to a true sensor [11] the particles have to be immobilized. In this particular case, a matrix which allows for the swelling of

the particle without losing its immobilizing feature is of great importance. Due to the ratiometric measurement principle, a small loss of particles will not influence the measurements, but the overall sensor lifetime will be much shorter if a great portion of the particles is washed out over time. Hydromed D6, a hydrogel based on polyurethane, allows for the entrapment of the particles. It is a block copolymer with both hydrophobic and hydrophilic groups, is not crosslinked and has a water content of 80%. While this is probably not the best immobilization matrix, it enabled us to perform a first series of tests, to see whether the swelling is reversible and these particles could be used as sensors.

The sensor film is prepared by mixing a solution of the particles in ethanol with a solution of the hydrogel in ethanol/water 9/1. This cocktail is then knife coated onto a PET substrate and left to dry overnight, resulting in a thin film (30  $\mu\text{m}$ ) in which the particles are entrapped (see Fig. S8 in the Supporting Information). This substrate can be mounted in a flow through cell and solutions containing different amounts of sugar can be passed by. Figure 6 shows the response of the particles AM1 to changing fructose concentrations at pH 8.5 with an ionic strength of 154 mM.

In the first segment of the measurement, 6 and 0 mM fructose are measured alternately, and a quick and reproducible response ( $t_{90}$  for swelling and shrinking: 1.5 min) of the particles can be measured. Also, the complete reversibility of the swelling can be seen in this measurement. As soon as the solution was changed to 0 mM fructose, the signal ratio and therefore the particle size began to change and returned to its initial value. At 3 mM fructose the  $t_{90}$  is rising to 4.5 min. With fewer generated negative phenylboronic acid ions, the swelling takes longer because of the constraining force the hydrogel exhibits. The time for the shrinking remains at 1.5 min. The particles are immobilized via spatial hindrances in the hydrogel and thus prevented from leeching out of the



**Fig. 6** Response of the immobilized particles to fructose. The swelling is reversible and different fructose concentrations result in different intensity ratios. The label in the graphic shows the time of change to the given concentration ( $\lambda_{\text{ex}}$ : 470 nm; pH 8.5; ionic strength 154 mM)

system. These hindrances can be imagined to be obstructive to the swelling of the particles and beneficial for the shrinking of the particles. This can be seen in the fact that the shrinking time is independent on the fructose concentration. It seems as if the shrinking of the particles is aided by the immobilization once the “pressure” of the particles is not present any more.

The response to inclining and declining fructose concentrations can be seen in the last segment of Fig. 6. The particles respond to the changing fructose concentrations with fairly stable equilibrium sizes. Again, moderate response times for the swelling and fast response times for the shrinking can be observed. The dynamic range is somewhat limited but could be expanded when using particles with different phenylboronic acid contents and/or other ratios of the dyes. The immobilized particles also respond to glucose, but like the experiments in solution showed, a ten times higher concentration of glucose is needed, to get a similar response of the particles.

## Conclusion and outlook

We have synthesized fluorescent stimuli responsive nanoparticles which respond to sugar with a fast swelling due to a reaction of the sugar with phenylboronic acid. By reducing the amount of phenylboronic acid in the particle compared to previous reports, the response time for the swelling could be drastically reduced to 1.5 min in the case for fructose at pH 7.5 taking advantage from nanotechnology. Different sugar concentrations gave different equilibrium sizes with an almost linear calibration curve, and the swollen particles showed a stable fluorescence intensity ratio for at least 12 h. When using a phenylboronic acid monomer with a  $pK_a$  of 7.9 measurements of glucose at physiological pH were possible. By immobilizing the particles in a hydrogel matrix, we were able to demonstrate that the swelling process is reversible and the particles could be used in a sensor.

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