

Supporting Information for:

Boronic acid-modified poly(amidoamine) dendrimers as sugar-sensing materials in water

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Materials

D-ribose (98%) and PAMAM dendrimer, ethylenediamine core generation 3.0 solution (20 wt.% in methanol) were purchased from Aldrich. 4 Å molecular sieves were purchased from EMD and regenerated in an oven overnight at 120°C before use. Alizarin Red S was obtained from Acros, D-(-)-fructose and D-glucose from J. T. Baker, 6,7-Dihydroxy-4-methylcoumarin from Alfa-Aesar, methanol from BDH. 4-Formylphenylboronic acid was purchased from Oakwood Chemical. 3-Formylphenylboronic acid (97%) and 2-formylphenylboronic acid (97%) were purchased from Matrix Scientific. Sodium borohydride was used as received from TCI. Dimethyl sulfoxide-D₆ and CD₃OD (D, 99.9%) were obtained from Cambridge Isotope Laboratories. D-(-)-galactose was used as received from Riedel-de Haën AG. Dialysis membranes (6k – 8k Da MW cutoff) were obtained from Spectra/Pro and regenerated in DI water for 3 minutes before use. 3-(Cyclohexylamino)propanesulfonic acid (CAPS, 99.3%) was obtained from Chem-Impex Inc. 4-(2-Hydroxyethyl)piperazin-1-ylethanesulphonic acid (HEPES) was obtained from IBI Scientific. All materials were used as received unless otherwise noted.

Techniques

NMR Spectra (¹H, 360 MHz) were recorded on a Varian Unity Inova RF console in CD₃OD or DMSO-d₆ at 25°C. Chemical shifts are reported as δ (ppm) using the solvent peak as the reference standard.

The MALDI-TOF data were recorded on a Bruker Ultraflex I TOF/TOF workstation using positive ionization method. MALDI-TOF samples (4.34 mg/mL) were prepared in MeOH with sinapinic acid (SA, 10 mg/mL, 70/30 CAN/water, 0.1% TFA) as the matrix. Equal volumes of the matrix and dendrimer solution were mixed and 1 μL of the mixture was spotted on a MALDI plate.

Dynamic light scattering (DLS) experiments were carried out on a Malvern Zeta Sizer Nano station model LEN3600. Dye alizarin red S (ARS) and **PAMAM-*m*-ba** were dissolved in 50 mM HEPES buffer. The experimental conditions were as follows: [ARS] = 5.208 × 10⁻⁵ M. [PAMAM-*m*-ba] = 1.125 × 10⁻⁴ M. The refractive index was set to *n*_{20/D} 1.367.

All optical spectroscopy experiments were carried out in aqueous solutions buffered to pH 7.4 with 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, 50 mM), to or pH 10.0 with 3-(cyclohexylamino)propanesulfonic acid (CAPS, 50 mM) using a freshly calibrated glass combined electrode to monitor the pH. The pH of the working solutions was adjusted prior to use by addition of NaOH or HCl solutions and spot-checked during titration to make sure that it had not drifted away from the desired value.

UV-Vis absorption spectra were acquired on a HP 8452A diode array UV-Vis spectrophotometer, recording spectra over the range 230-800 nm with a resolution of 2 nm; the cuvette holder temperature (25°C) was controlled by an external circulating water bath.

Benchtop fluorescence measurements were carried out with a ISS PC1 spectrofluorimeter, with manual calibrated slits and high-aperture Glan- Thompson calcite polarizers in the excitation and emission channels to measure steady-state fluorescence anisotropy. Excitation was carried out using a broad spectrum high pressure xenon lamp (CERMAX, 300W). Excitation correction was performed through a rhodamine B quantum counter with a dedicated detector. Excitation intensity was controlled by a manually operated iris (open/closed only). Detection was through a Hamamatsu red-sensitive PMT. Experimental temperature (25°C) was controlled by external circulating water bath.

Titration design: Binding experiments were carried out by adding aliquots of a “titrant” solution containing boronic acid-modified dendrimer to a “cuvette” solution containing the dye under study. In addition to the polymer, the titrant solution also contained the dye under study at the same concentration as the cuvette solution, to prevent any change in the concentration of the dye during the course of the additions. Using this method, the concentration of dye and dendrimer remained constant throughout the titration and dilution artefacts were eliminated. Titrations on benchtop instruments were carried out by addition of multiple aliquots of titrant solution to a constant volume of cuvette solution. Titrant and cuvette solutions were made fresh for each experiment.

Displacement experiments were carried out similarly, using two separate working solutions: a “titrant” and a “cuvette” solution. A cuvette solution contained both the dye and the receptor **PAMAM-*m*-ba** and **PAMAM-*o*-ba** at the appropriate ratio to form the desired bound dye complex. The titrant solution contained dye and dendrimer at the same concentration as the cuvette solution, and it also contained a displacer sugar at an appropriate concentration to carry out the titration.

Array sensing: Spectroscopic data for array sensing experiments were acquired on a BioTek Synergy II multimode microwell plate reader, capable of measuring absorbance spectra (through a monochromator), and steady-state fluorescence intensity and anisotropy (through bandpass filter sets). The sample compartment in this instrument is electrically thermostatted.

Experiments were laid out by hand using Eppendorf Research multichannel pipettors and disposable plastic tips into microwell plates with clear bottom for UV and fluorescence (Greiner BioOne), in 384-well configuration. The plates were made of non-treated (medium binding) polystyrene with black walls (to minimize scattered light) and clear flat bottoms. Each well contained exactly 100 μ L of solution. In addition to sample points, the plates were also set up to contain reference (dye only) and blank (buffer only) wells, to use in cross-referencing / blanking of the acquired data.

The data acquired using the BioTek microwell plate reader included single-wavelength absorbance, non-polarized fluorescence emission, and fluorescence polarization measurements. In the case of fluorescence, the gain was adjusted automatically so that the highest reading from each plate reached 85% of the instrument’s full scale. Both absorbance and fluorescence emission raw data points were blanked by subtracting the average reading for the wells containing buffer.

Absorbance was collected at the following wavelengths: 334, 362, 380, 384, 510, and 700 nm. Fluorescence intensity was collected in the following channels ($\lambda_{\text{ex}}/\lambda_{\text{em}}$): 380/450 and 516/580 nm. Fluorescence anisotropy was collected in the channels ($\lambda_{\text{ex}}/\lambda_{\text{em}}$): 380/450 nm.

The data for qualitative and quantitative analyses were then processed using a Linear Discriminant Analysis (LDA) algorithms implemented in-house using the *Mathematica* software package (see below).

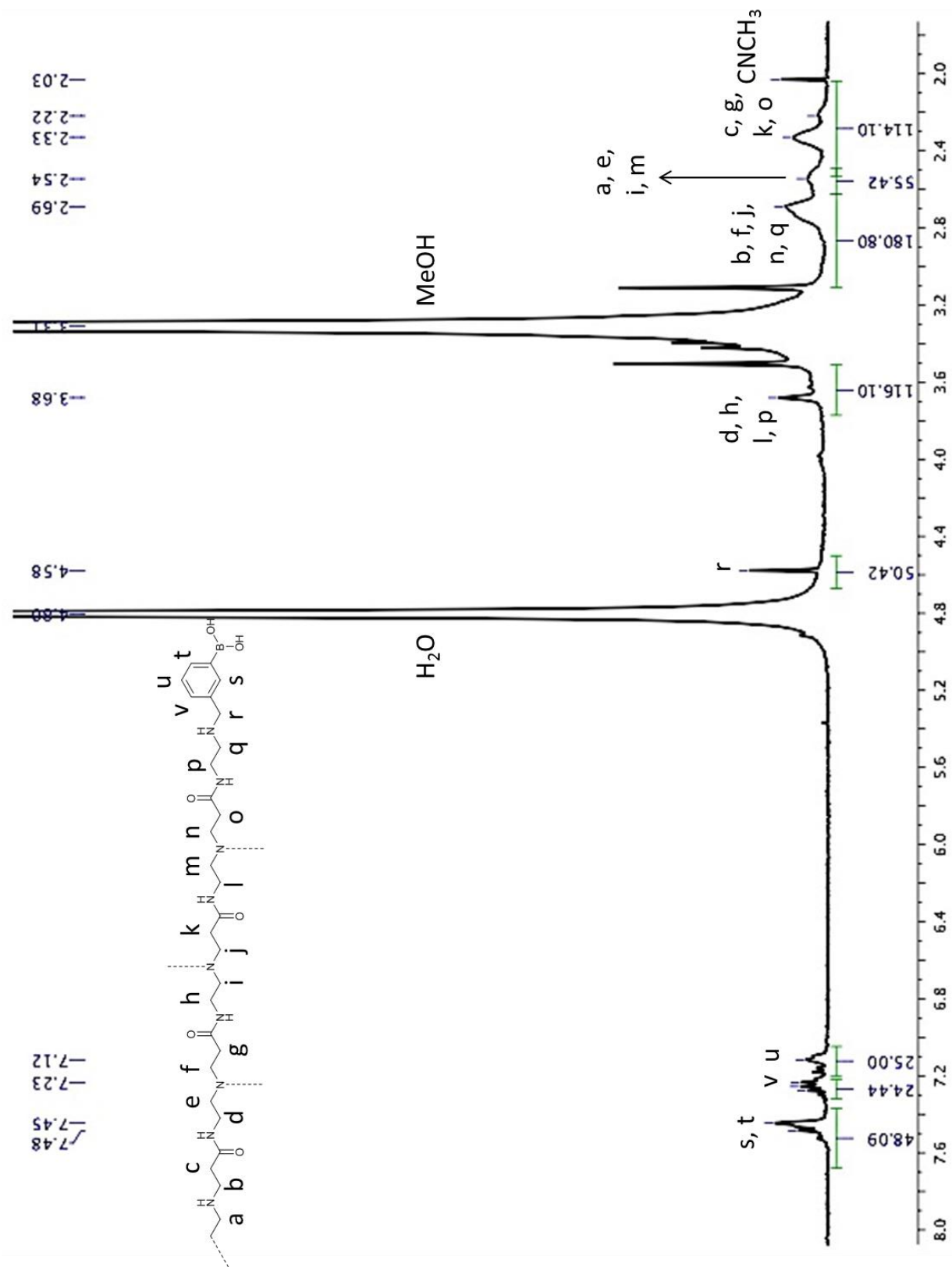


Figure S-1. ^1H NMR spectrum of PAMAM-*m*-ba

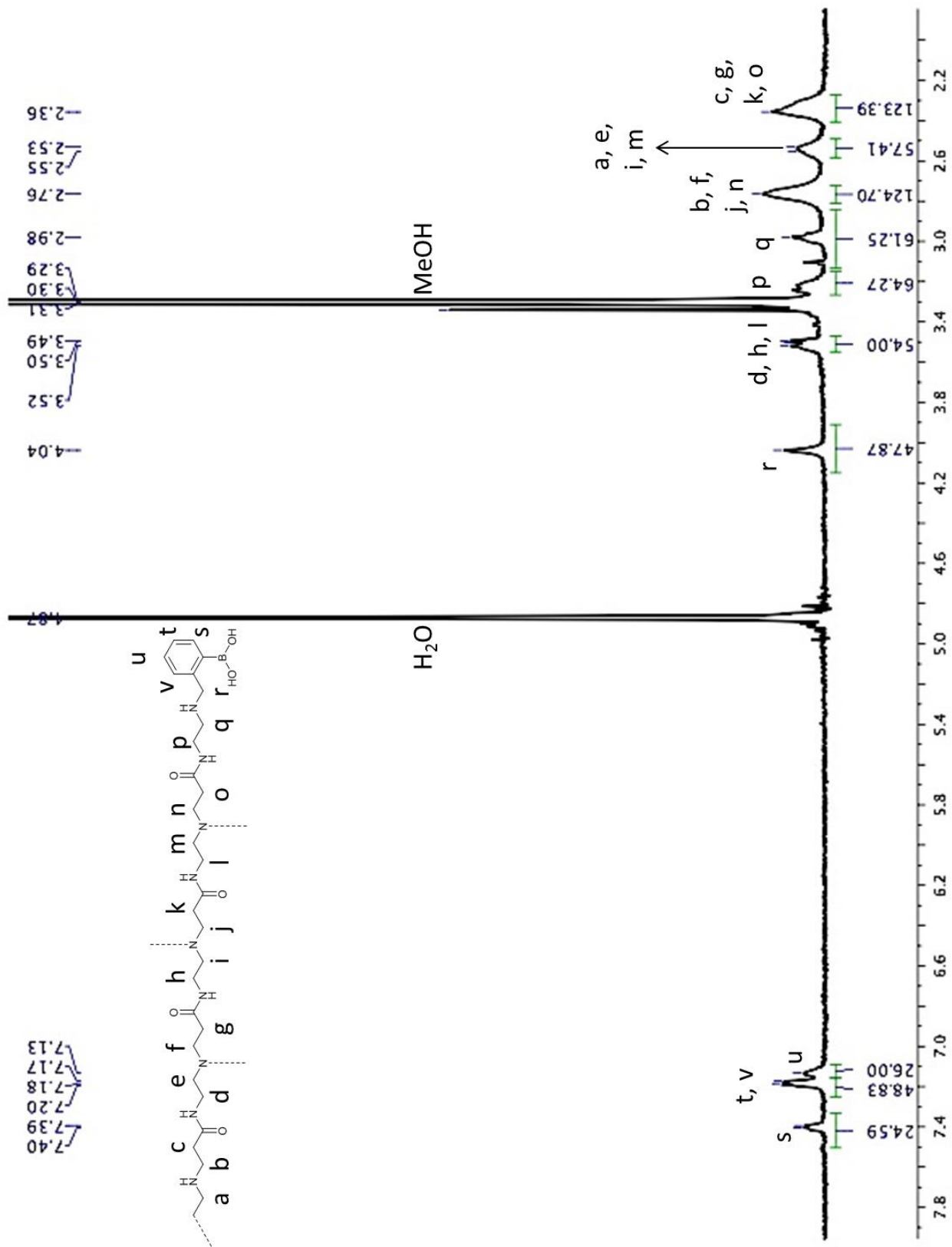


Figure S-2. ¹H NMR spectrum of PAMAM-*o*-ba

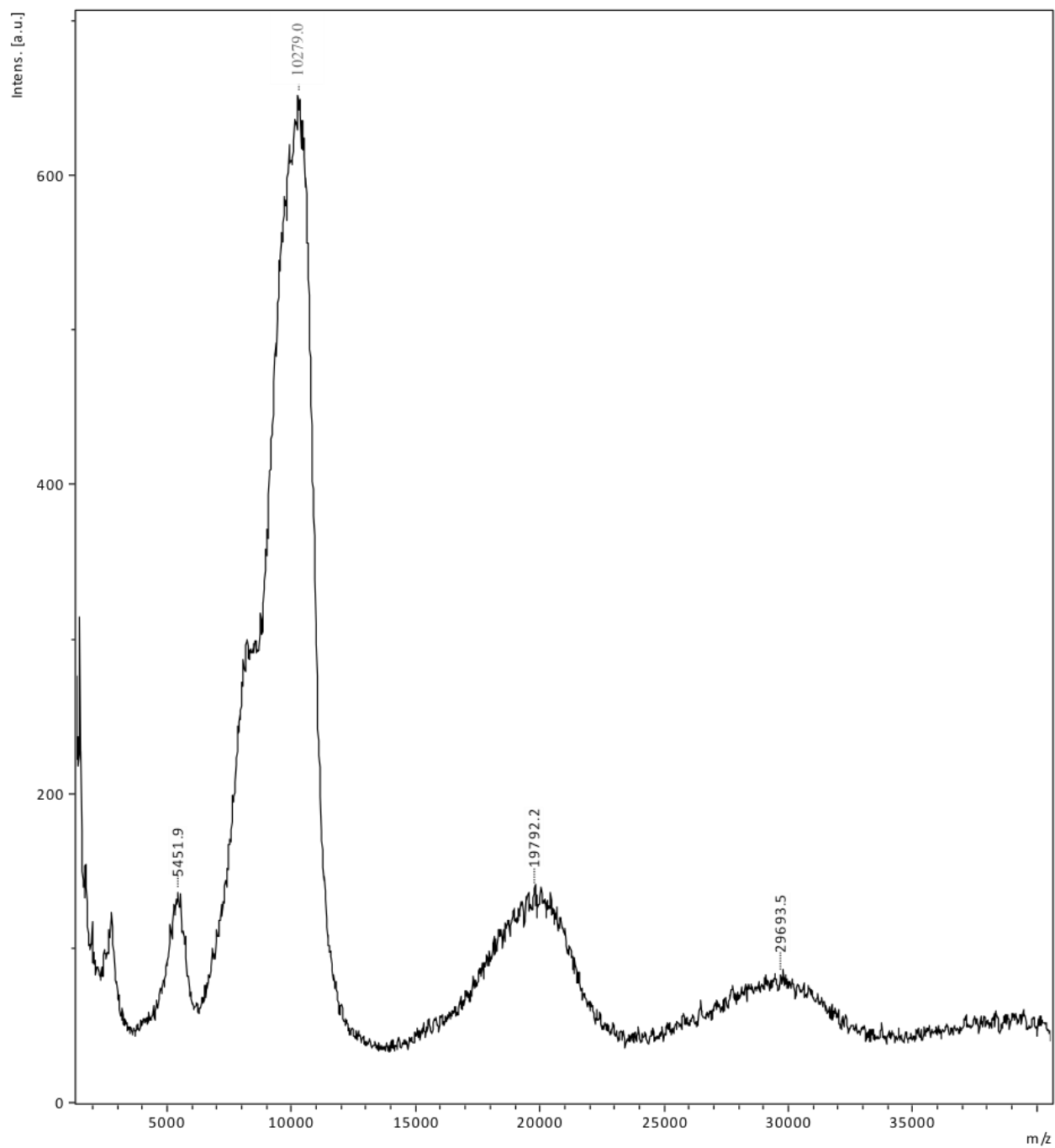


Figure S-3. MALDI-TOF spectrum of PAMAM-*m*-ba

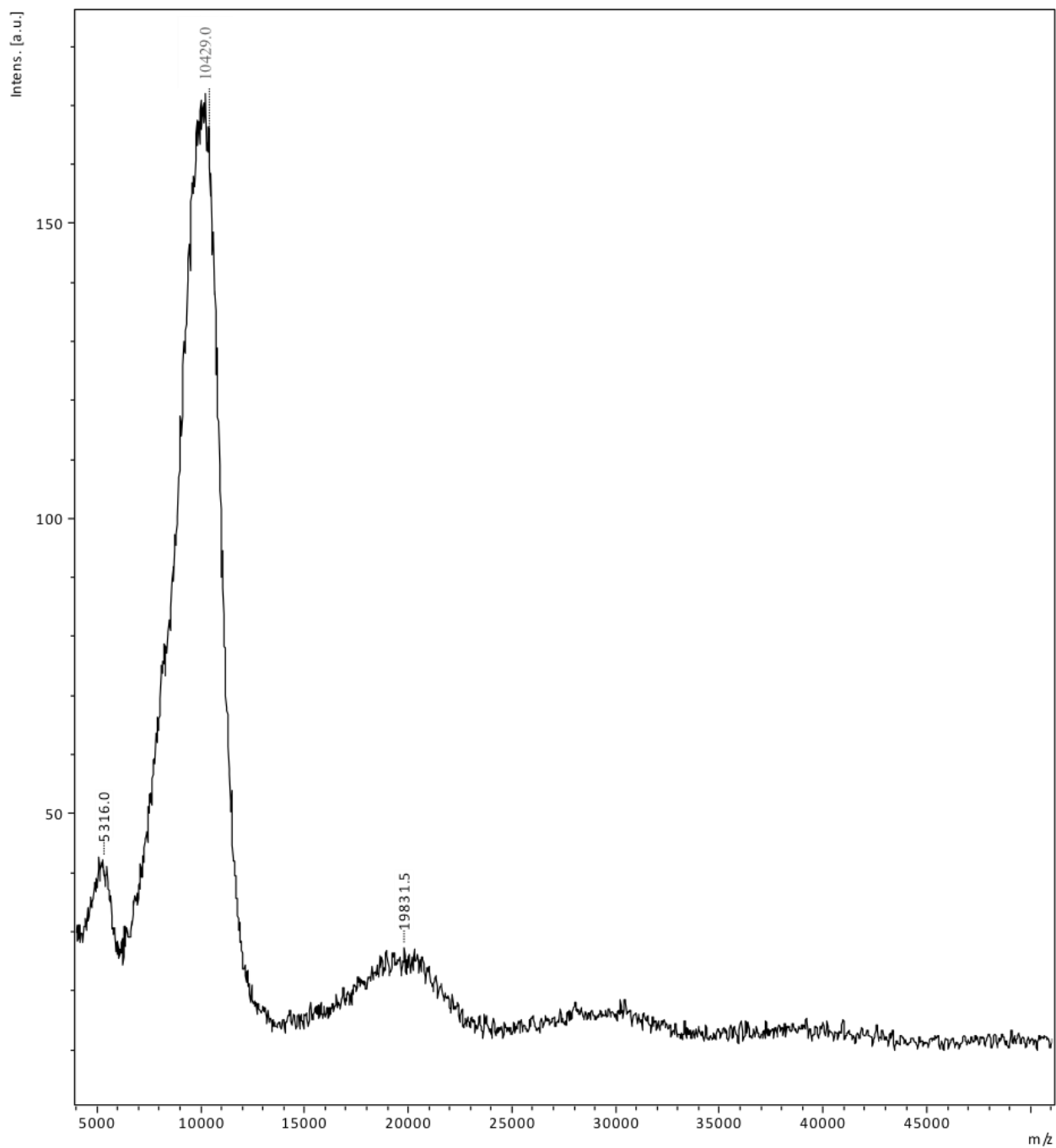


Figure S-4. MALDI-TOF spectrum of PAMAM-*o*-ba

Additional results from binding and displacement studies

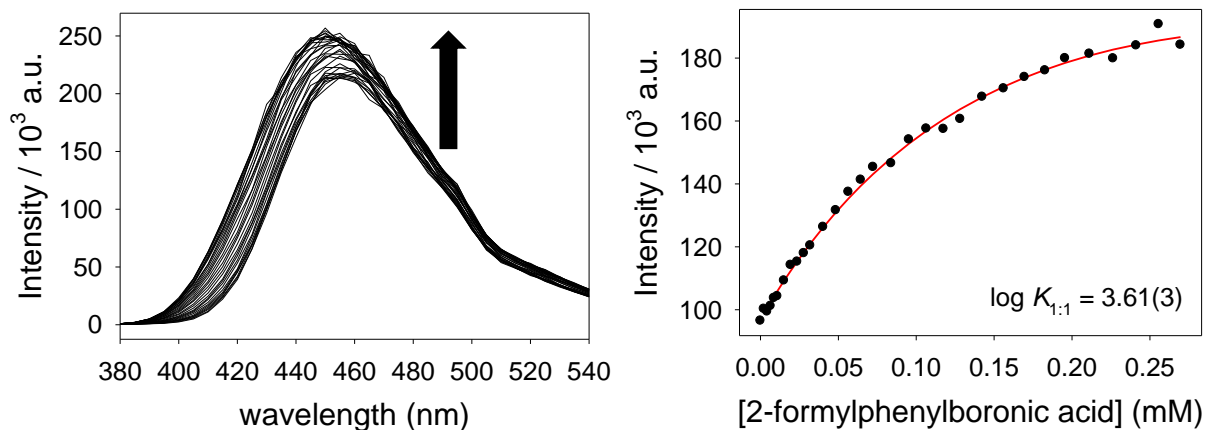


Figure S-5. (Left) Fluorescence intensity increased as 2-formylphenylboronic acid was titrated into a solution of **ML** dye. (Right) The titration profile at 430 nm, together with the results from the fit to a 1:1 binding model (red). $T = 25\text{ }^{\circ}\text{C}$; $\text{pH} = 7.4$ in water (50 mM HEPES); $[\text{ML}] = 1.057 \times 10^{-5}\text{ M}$; $\lambda_{\text{exc}} = 362\text{ nm}$; $\lambda_{\text{em}} = 430\text{ nm}$.

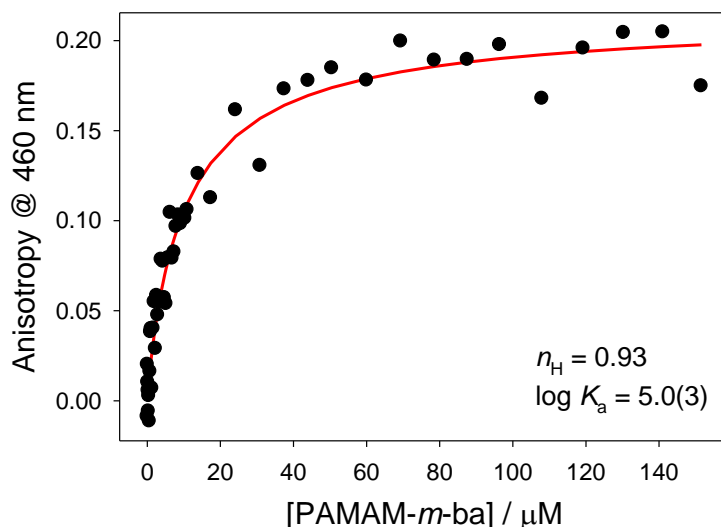


Figure S-6. Binding isotherm obtained from addition of PAMAM-*m*-ba to **ML**. The experimental data was fit to a modified Hill isotherm $r = (r_{\text{max}} - r_{\text{min}}) [\text{PAMAM-o-ba}]^n / ([\text{PAMAM-o-ba}]^n + K_d^n) + r_{\text{min}}$. $T = 25\text{ }^{\circ}\text{C}$; $\text{pH} = 7.4$ in water (50 mM HEPES); $[\text{ML}] = 1.057 \times 10^{-5}\text{ M}$; $\lambda_{\text{exc}} = 362\text{ nm}$; $\lambda_{\text{em}} = 450\text{ nm}$.

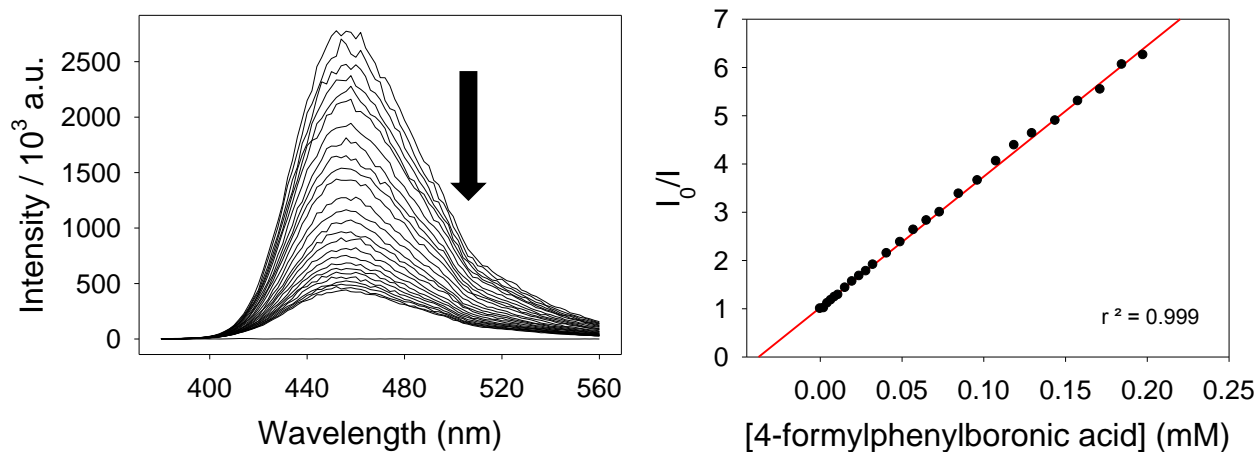


Figure S-7. (left) Quenching effect of 4-formylphenylboronic acid to ML; (right) Stern-Volmer plot: I_0/I vs. [boronic acid] and corresponding linear fit. $T = 25\text{ }^\circ\text{C}$; $\text{pH} = 7.4$ in water (50 mM HEPES); $[\text{ML}] = 1.057 \times 10^{-5}\text{ M}$; $\lambda_{\text{exc}} = 362\text{ nm}$; $\lambda_{\text{em}} = 450\text{ nm}$.

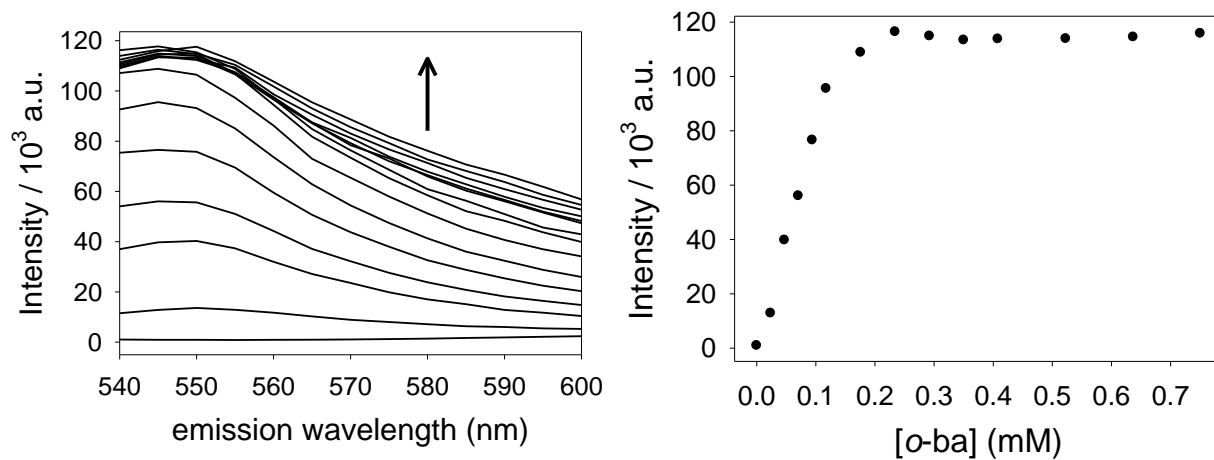


Figure S-8. Direct binding of PAMAM-*o*-ba and alizarin red S (ARS) at pH 7.4 monitored by fluorescence spectroscopy. $T = 25\text{ }^\circ\text{C}$; $\text{pH} = 7.4$ in 50 mM HEPES; $[\text{ARS}] = 5.257 \times 10^{-5}\text{ M}$; $\lambda_{\text{exc}} = 518\text{ nm}$; $\lambda_{\text{em}} = 545\text{ nm}$.

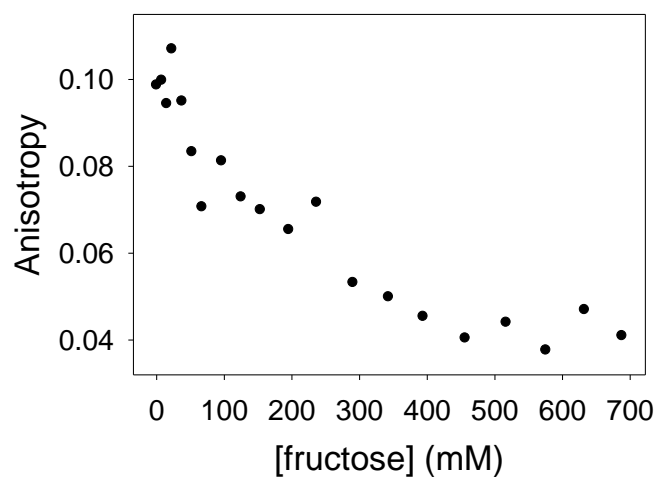


Figure S-9. Fructose binding to **PAMAM-*o*-ba** at pH 7.4, monitored by dye displacement using the [**PAMAM-*o*-ba** • **ML**] system (**ML** = 4-methylesculetin). T = 25 °C; pH = 7.4 in water (50 mM HEPES); [**ML**] = 1.034×10^{-5} M; [**PAMAM-*o*-ba**] = 1.363×10^{-5} M; λ_{exc} = 362 nm; λ_{em} = 450 nm.

Multivariate data analysis and pattern sensing:

In order to evaluate the discriminatory power of the **PAMAM-ba** molecular probes, Linear Discriminant Analysis (LDA) was used as a multivariate approach to simplify and interpret the datasets collected from multiwell plate experiments. LDA is a statistical data treatment method that is in common use for reinterpretation of a multidimensional data set. All multivariate analyses were performed using code developed in-house using the *Mathematica* software system (release 10.3) published by Wolfram Research Inc.

As a first step towards data analysis, each variable in the raw experimental data set was mean-centred and standardized, a common practice in multivariate data analysis in the case of input data spanning very different dynamic ranges, as is the case in e.g. absorbance and fluorescence intensity raw data in our set.

The resulting standardized data set was subjected to LDA. The resulting score plot is shown in the main manuscript; the corresponding loading plot depicted in Figure S-7 reports on the contribution of each one of the 40 instrumental variables to the overall discriminatory power of the sensing system

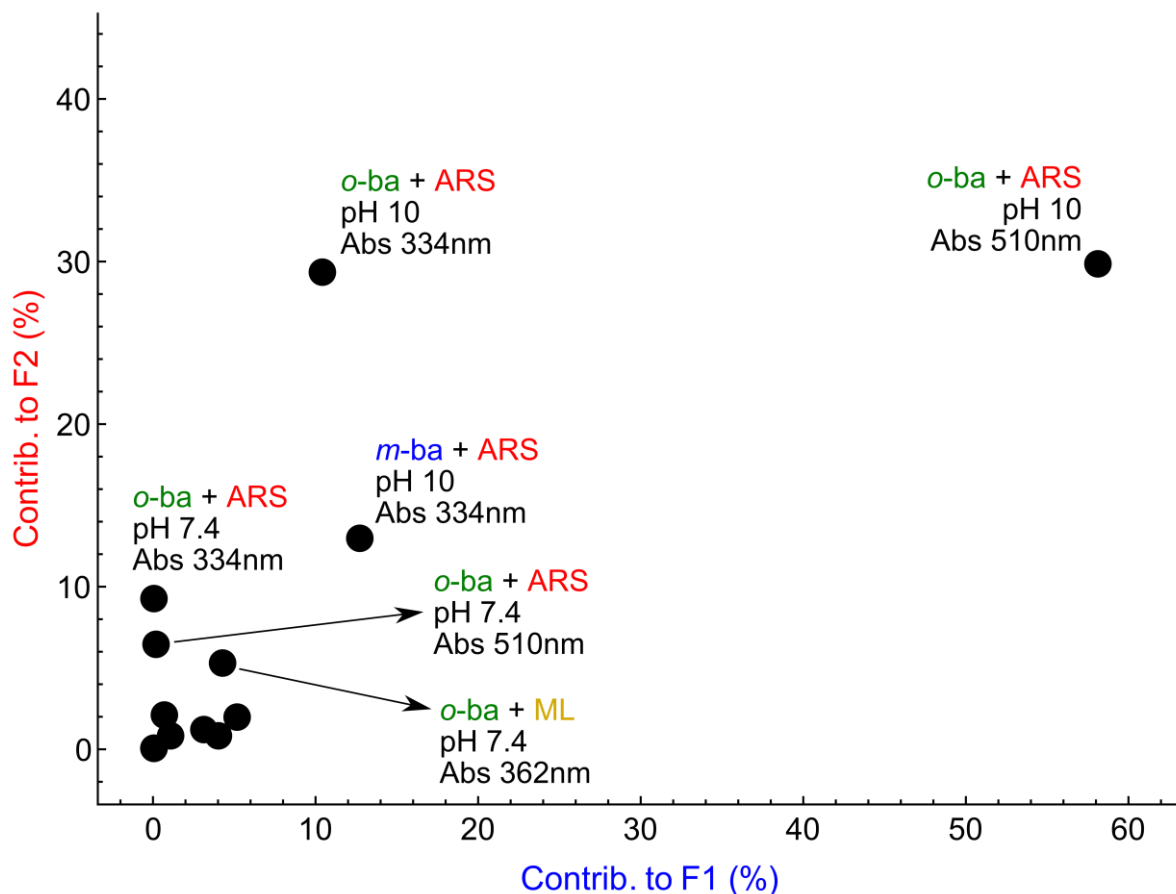


Figure S-10. Loading plot obtained from LDA multivariate analysis; ARS = alizarin red S; ML = 4-methylesculetin; *o*-ba = PAMAM-*o*-ba; *m*-ba = PAMAM-*m*-ba; Abs = absorbance value. Instrumental measurements contributing less than ca. 5% to either factor were included, but not individually labelled.

The loadings report on the contribution of each original instrumental variable to the two factors we selected for plotting. Inspection of the loading plot reveals that among the two polymeric receptors we synthesized, the **PAMAM-*o*-ba** receptor seems to contribute the most to the differentiation of the selected sugars.

All major contributions are labelled in Figure S-10; further non-insignificant contributions are made by other instrumental factors; however, these are individually low, and have not been labelled in the loading plot. All contributions are reported in Table S-1 below.

Receptor	Dye	pH	Type	Wavelength	F1%	F2%
PAMAM- <i>o</i> -ba	ARS	10.0	Abs	510 nm	58.2	29.8
PAMAM- <i>m</i> -ba	ARS	10.0	Abs	334 nm	12.7	13.0
PAMAM- <i>o</i> -ba	ARS	10.0	Abs	334 nm	10.5	29.3
PAMAM- <i>m</i> -ba	ARS	10.0	Abs	510 nm	5.2	2.0
PAMAM- <i>o</i> -ba	ML	7.4	Abs	362 nm	4.3	5.2
PAMAM- <i>m</i> -ba	ARS	7.4	Abs	510 nm	4.0	0.8
PAMAM- <i>m</i> -ba	ARS	7.4	Abs	334 nm	3.1	1.2
PAMAM- <i>m</i> -ba	ML	10.0	Abs	384 nm	1.2	0.8
PAMAM- <i>m</i> -ba	ML	7.4	Abs	362 nm	0.8	2.1
PAMAM- <i>o</i> -ba	ARS	7.4	Abs	510 nm	0.2	6.4
PAMAM- <i>o</i> -ba	ARS	7.4	Abs	334 nm	0.0	9.3
PAMAM- <i>m</i> -ba	ML	10.0	I	380/450 nm	0.0	0.0
PAMAM- <i>m</i> -ba	ML	7.4	I	380/450 nm	0.0	0.0
PAMAM- <i>o</i> -ba	ML	7.4	I	380/450 nm	0.0	0.0
PAMAM- <i>m</i> -ba	ARS	10.0	I	516/580 nm	0.0	0.0
PAMAM- <i>m</i> -ba	ARS	7.4	I	516/580 nm	0.0	0.0
PAMAM- <i>o</i> -ba	ARS	10.0	I	516/580 nm	0.0	0.0
PAMAM- <i>o</i> -ba	ARS	7.4	I	516/580 nm	0.0	0.0
PAMAM- <i>m</i> -ba	ML	10.0	Pol	380/450 nm	0.0	0.0
PAMAM- <i>m</i> -ba	ML	7.4	Pol	380/450 nm	0.0	0.0
PAMAM- <i>o</i> -ba	ML	7.4	Pol	380/450 nm	0.0	0.0

Table S-1. Loadings for all instrumental variables measured. Abs = absorbance; I = fluorescence intensity; Pol = fluorescence anisotropy (wavelength pairs correspond to excitation and emission filters); $F_n(\%)$ is the percentage of the information carried by factor n that is due to that instrumental measurement.

In addition, absorbance measurements were found to be more important in general than fluorescence emission. In particular, absorbance values associated with the ARS dye were found to be the most information rich, although the 4-methylresorcinol dye also contributes, in particular to the second factor.

Between the two pH values tested in this preliminary attempt, measurements at pH 10 seem to carry more information than those conducted at neutral pH. That notwithstanding, the contribution of measurements at pH 7 is still significant, as attempting their removal has a visible negative impact on sugar differentiation.