Supporting Information for:

Carbohydrate sensing using water soluble poly(methacrylic acid)-*co*-3-(acrylamide)phenylboronic acid co-polymer

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Materials

3-acrylamidophenylboronic acid (AAPBA) and D-(-)-ribose (98%) were purchased from Aldrich. D-trehalose (99%) was purchased from Acros. D-(-)-fructose was from J. T. Baker. 6,7-Dihydroxy-4-methyl-coumarin (97%), sucrose (99%), D-(+)-maltose monohydrate (95%), α-D-lactose monohydrate (ACS grade) and D-(+)-galactose (98%) were purchased from Alfa-Aesar. *tert*-Butylmethacrylate (tBMA) (Aldrich) and 1,4-dioxane (Fisher Scientific) were distilled under low pressure before copolymerization. 2,2'-Azobis(2-methylpropionitrile) (AIBN) (Aldrich) was recrystallized from methanol before use. D-(+)-glucose (>98.0%) and hematoxylin hydrate (>97.0%) were purchased from TCI. Cyanidin chloride was purchased from Extrasynthese. Dimethylsulfoxide (DMSO) (ACS grade) was purchased from EMD. 4-(2-Hydroxy-ethyl)-piperazin-1-ylethanesulphonic acid (HEPES) was obtained from IBI Scientific. Dichloromethane (DCM) and trifluoroacetic acid (TFA) were purchased from Fisher Scientific. 3-Formyl¬phenyl¬boronic acid (97%) was purchased from Matrix Scientific. Ultrapure deionized water with resistivity of 0.055 μS/cm (18.2 Ω cm) was used in all experiments (Siemens). All materials were used as received unless otherwise noted.

Characterization of copolymer PMAA-*co*-AAPBA.

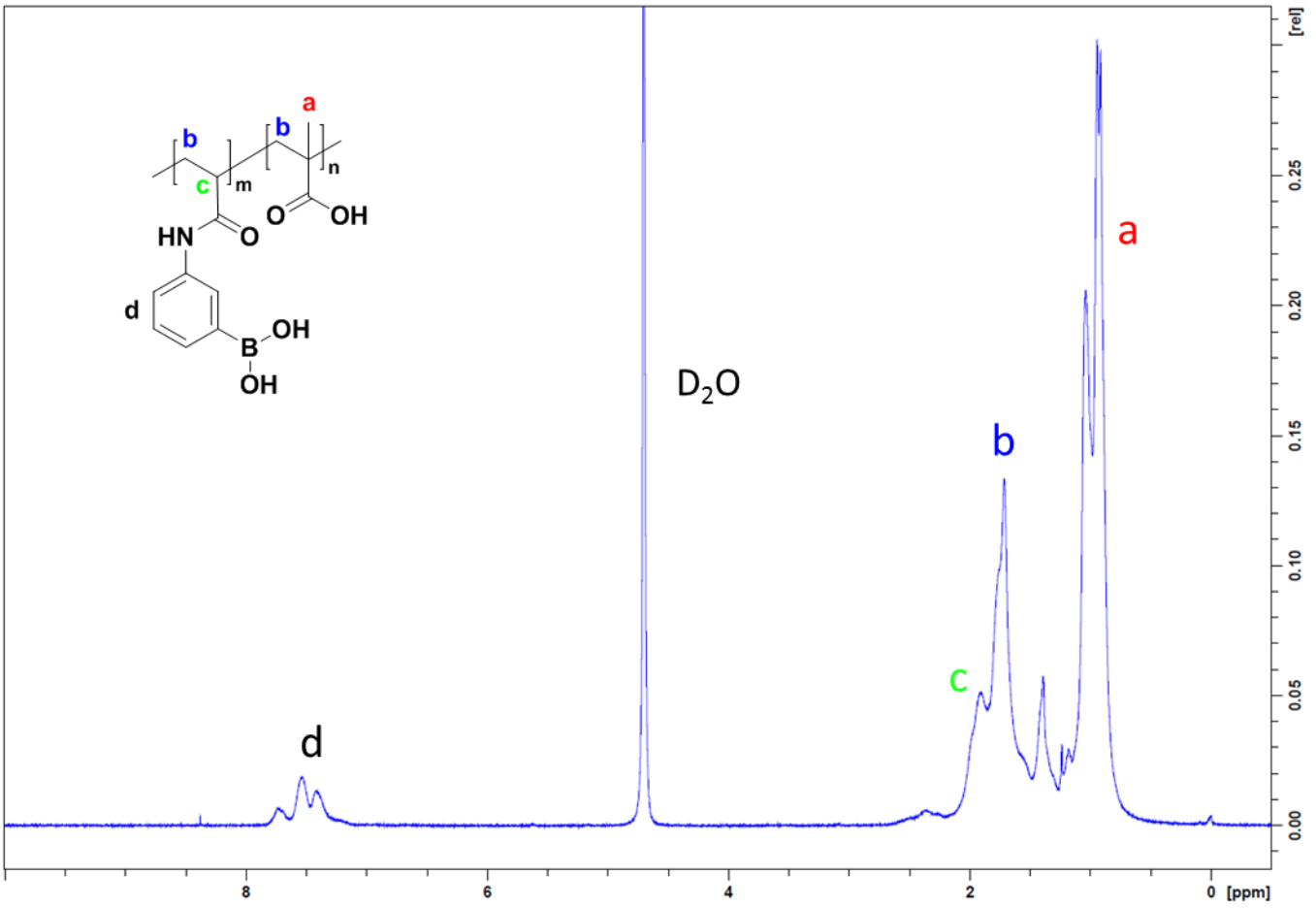


Figure S1 ^{1}H NMR spectrum of poly(methacrylic acid)-*co*-3-(acrylamido)phenylboronic acid (**PMAA-*co*-AAPBA**) containing 6% of 3-(acrylamido)phenylboronic acid units.

The fraction of 3-(acrylamido)phenylboronic acid-containing units (6%) was calculated as follows: $f = m/(m + n)$, where the m/n ratio was found from the integral intensities corresponding to the aromatic protons of the phenylboronic acid component (4 m; δ: 7.1 – 7.4 ppm) and the methylene, methine, and methyl protons (5 n + 7 m; δ: 0.5 – 2.0 ppm). The spectrum is taken in D_2O (δ: 4.5-4.7 ppm; Sigma-Aldrich).

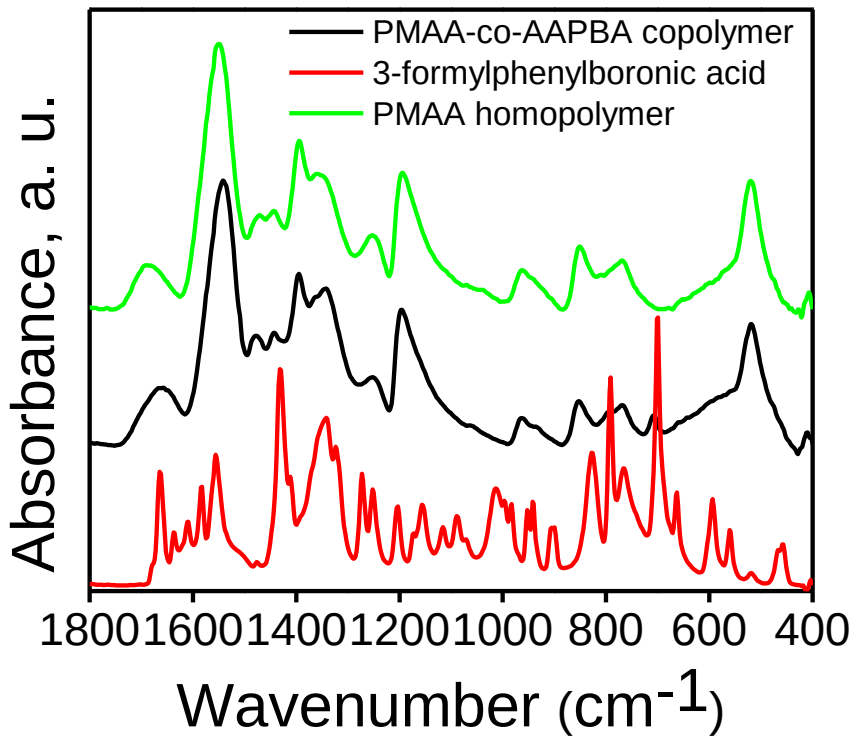


Figure S2 FTIR spectra of poly(methacrylic acid) homo-polymer (PMAA, green), poly(methacrylic acid)-*co*-3-(acrylamido)phenylboronic acid (PMAA-*co*-AAPBA) containing 6% of 3-(acrylamido)phenylboronic acid units (PMAA-*co*-AAPBA-6, black), and 3-(acrylamido)phenylboronic acid powder (AAPBA, red). FTIR spectra show that peaks corresponding to AAPBA and PMAA are present in the copolymer. Carboxyl groups for PMAA homopolymer and copolymer are present at 1562 cm^{-1} (antisymmetric) and at 1408 cm^{-1} (symmetric). PMAA-co-AAPBA was compared to 3-formylphenylboronic acid and the out-of-plane phenyl ring deformation peaks were present at around 650-696 cm^{-1}.

Techniques for spectroscopic binding experiments

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), 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 and spot-checked during titration to make sure that it had not drifted away from the desired value. All sugar and polymer solutions were prepared using this buffer. Dye stock solutions were prepared by first dissolving the dye in DMSO; these stocks were then diluted with HEPES buffer to the working concentration. The final concentration of DMSO in the cuvette was extremely low: 20 μL of this stock solution were diluted to 2.9 mL to generate the final working cuvette solution, for a final DMSO concentration in the working solution of 0.69% v/v.

Solutions were prepared using Eppendorf Research single-channel micropipettes with disposable tips, by addition of appropriate amounts of stock solutions to volumetric flasks, which were then brought up to volume.

Absorption spectra were first 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 temperature (25°C) was controlled by an external circulating water bath.

Fluorescence measurements were carried out with a ISS PC1 spectrofluorimeter. 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). Manual calibrated slits controlled the spectral resolution. Detection was through a Hamamatsu red-sensitive PMT. Cuvette temperature (25°C) was controlled by an external circulating water bath.

Binding titration design: Direct binding experiments (e.g. dye + polymer) were carried out by adding aliquots of a titrant solution containing boronic acid-modified polymers to a cuvette solution containing the dye under study. Titrations on benchtop instruments were carried out by addition of multiple aliquots of titrant solution to a cuvette solution. Titrant and cuvette solutions were prepared fresh for each experiment.

Displacement experiments (e.g. sugar + dye-polymer complex) were carried out similarly, using two separate working solutions: a titrant and a cuvette solution. The cuvette solution contained both the dye and the polymer **PMAA-AAPBA** at the appropriate ratio to form the desired "bound dye" complex. The titrant solution contained dye and polymer at the same concentration as the cuvette solution, in order to keep the concentration of dye constant, and it also contained a displacer sugar at an appropriate concentration to carry out the titration.

Measurements for 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 μL of solution. In addition to sample points, the plates also contained wells for reference (dye only) and blank (buffer only) samples, to use in cross-referencing / blanking the acquired data.

The data acquired using the BioTek microwell plate reader included single-wavelength absorbance and fluorescence emission. 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 were blanked by subtracting the corresponding average reading for the wells containing buffer.

Absorbance was collected at the following wavelengths: 380, 430, 450, 485, 556, 560, 570 and 700 nm. Fluorescence intensity was collected with the following filter combinations:

Table S1 Filter combinations used in fluorescence detection on the microwell plate reader

Excitation filter	**Emission filter**
380 ±10 nm	580 ±25 nm
516±10 nm	580 ±25 nm
485 ±10 nm	645 ±20 nm
560 ±20 nm	645 ±20 nm

Multivariate data analysis and pattern sensing

Linear Discriminant Analysis (LDA) was used as a multivariate approach to analyze the datasets collected from micro-well plate experiments and interpret the discriminatory power of the **PMAA-*co*-AAPBA** molecular probes. LDA is a data treatment method that is in common use for reinterpretation of multidimensional data sets. All multivariate analyses were performed using code developed in-house using the *Mathematica* software system (release 11.2) published by Wolfram Research Inc.

In the first step of data analysis, every variable in the raw experimental data was mean-centered 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 standardized data set was subjected to LDA to yield a transformed multidimensional data set as a function of LDA factors.

<u>First results using both **HT** and **CC** dyes:</u>

We first attempted the discrimination of the series of simple carbohydrates shown in Figure 1 of the main manuscript. In this case, we used all dyes showing a significant interaction with the **PMAA-*co*-AAPBA** polymer, resulting in the following sensing species: oxidized, stabilized **HT** dye (i.e. hematoxylin and hematein mixture in the presence of ascorbate as described); cyanidin chloride (**CC**) dye; [**PMAA-*co*-AAPBA•HT**] complex; and [**PMAA-*co*-AAPBA•CC**] complex. Absorption and fluorescence intensity acquisition were carried out on a multiwell plate reader as described above. Ten replicate samples were measured for each analyte. A total of 11 measurements (e.g. absorbance, fluorescence emission at varying wavelengths) was acquired for each replicate. Samples were laid out on black polystyrene 384 well plates with transparent bottom, together with relevant blanks and standards.

Once raw experimental measurements were acquired, we used linear discriminant analysis (LDA) to analyze the data set. The score plot is shown in Figure S5. The clusters of each sugar are slightly tighter than those obtained when we used **[PMAA-*co*-AAPBA • HT]** only as sensor; however, the LDA loadings (Table S2) indicate that **HT** and its complex are the most important contributors to sugar discrimination. In particular, the **CC** dye and its complex contribute little to nothing to the discrimination, so they were not used further.

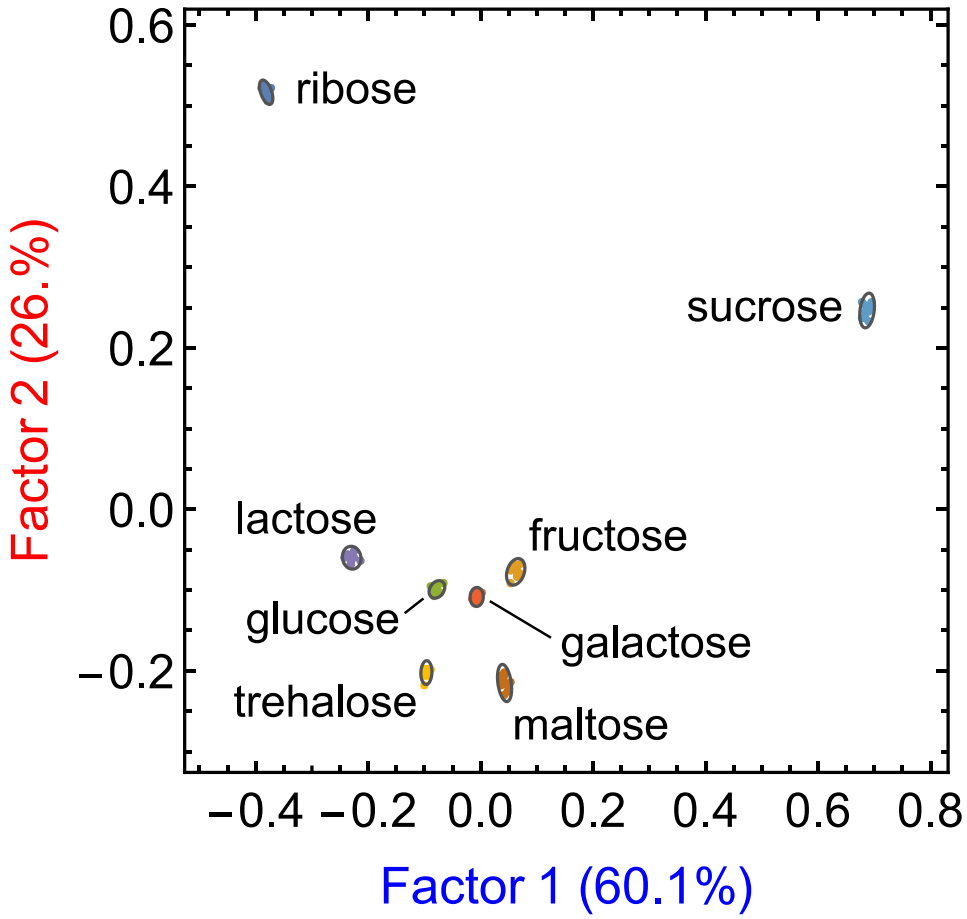


Figure S3 Two-dimensional LDA score plot on the data set obtained using **PMAA-*co*-AAPBA** complexes with both cyanidin (**CC**) and hematoxylin / hematein (**HT**) dyes. Experimental conditions: [**PMAA-*co*-AAPBA]** = 1.35 g/L, [**HT**] = 121.8 μM, [**CC**] = 22.93μM, [sugar] = 0.3 M, [ascorbate] = 596.2 μM, T = 25ºC, pH 7.4 HEPES (50 mM) aqueous buffer.

Table S2 LDA loading table for the first three factors from the analysis of data collected for the discrimination of small sugars.

Receptor	Type	Wavelengths	F1	F2	F3
[**HT** • PMAA-*co*-AAPBA]	absorbance	556 nm	14	14	12
		560 nm	4	11	15
		485 nm	0	3	1
		570 nm	2	1	0
		430 nm	0	1	1
		380 nm	0	0	0
		450 nm	0	0	0
	fluo	516/580 nm	0	0	2
		560/645 nm	0	0	2
		380/580 nm	0	0	0
		485/645 nm	0	0	0
Hematoxylin (**HT**)	absorbance	560 nm	17	30	20
		570 nm	14	25	23
		556 nm	27	1	4
		450 nm	2	3	2
		430 nm	1	1	3
		485 nm	0	0	4
		380 nm	3	0	0
	fluo	560/645 nm	1	0	0
		380/580 nm	0	0	0
		485/645 nm	0	0	0
		516/580 nm	0	0	0
[**CC** • PMAA-*co*-AAPBA]	absorbance	556 nm	1	4	4
		560 nm	0	2	3
		570 nm	2	1	0
		430 nm	0	0	1
		380 nm	0	0	0
		450 nm	0	0	0
		485 nm	0	0	0
	fluo	560/645 nm	0	0	1
		380/580 nm	0	0	0
		485/645 nm	0	0	0
Cyanidine chloride (**CC**)	absorbance	570 nm	6	1	0
		485 nm	1	1	0
		430 nm	0	1	0
		450 nm	1	0	0
		556 nm	1	0	0
		380 nm	0	0	0
		560 nm	0	0	0
	fluor	380/580 nm	0	0	0
		485/645 nm	0	0	0
		560/645 nm	0	0	0

HT = partially oxidized, ascorbate stabilized hematoxylin / hematein dye.

CC = cyanidin chloride dye

Factor 1 (F1) is mainly influenced by the absorption at 556 nm of the **HT** dye as well as its complex with the **PMAA-*co*-AAPBA** polymer.

Factor 2 (F2) mostly reflects effects of absorption at 560 nm and 570 nm of the **HT** dye and its complex with the **PMAA-*co*-AAPBA** polymer.

Results from simplified sensing system using only **HT** dye:

Once we realized that the contribution of **CC** dye species was small, we proceeded to simplify the sensing system by eliminating that dye and its complex. We therefore reduced the system to a single sensors, the [**PMAA-*co*-AAPBA•HT**] complex. We used the same analytes as in the previous attempt (Figure 1 of the main manuscript).

Absorption and fluorescence intensity acquisition were carried out on a multiwell plate reader as described above. Ten replicate samples were measured for each analyte. A total of 11 measurements (e.g. absorbance, fluorescence emission at varying wavelengths) was acquired for each replicate. Samples were laid out on black polystyrene 384 well plates with transparent bottom, together with relevant blanks and standards.

Here as well, once raw experimental measurements were acquired, we used linear discriminant analysis (LDA) to analyze the data set. The score plot is shown below (**Figure S4**) and in the main manuscript (Figure 10). The corresponding factor loadings are shown in **Table S3**, which reports on the contribution of each instrumental measurement to the first two factors that were selected for plotting.

The clusters of each sugar are slightly larger than those obtained above, but the system is much more practical because it uses only one sensor.

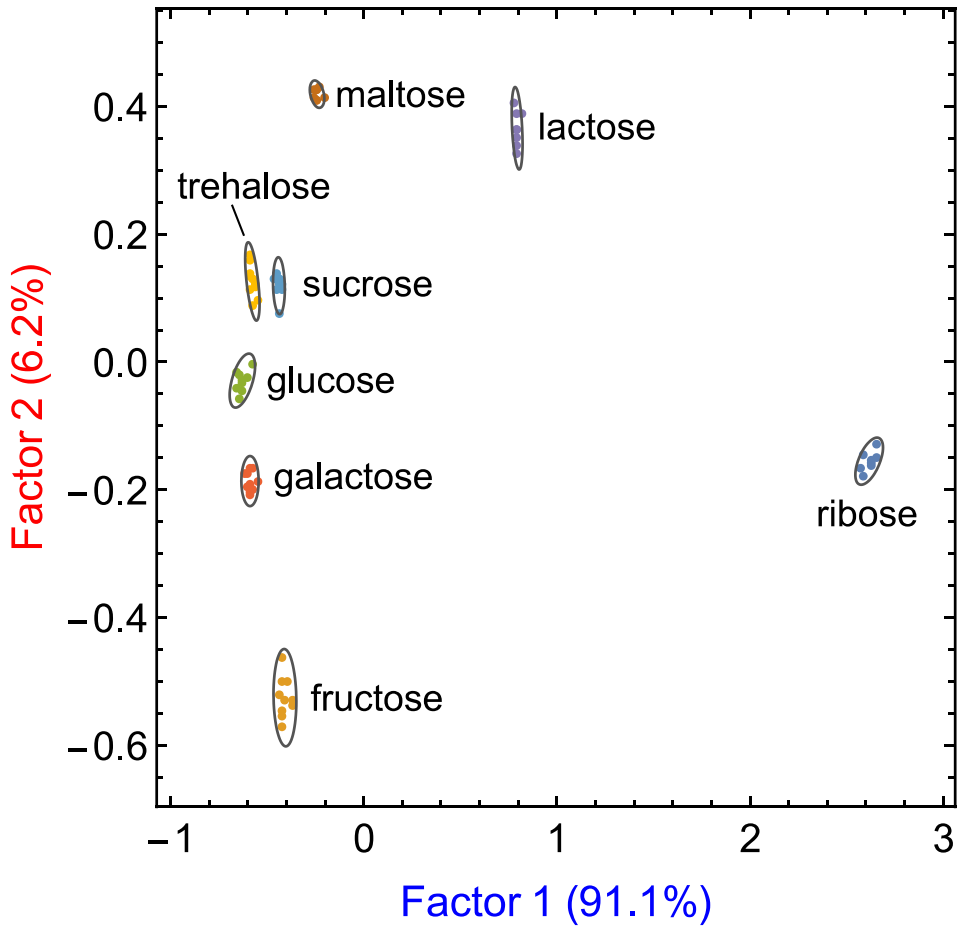


Figure S4 Two-dimensional score plot from the results of linear discriminant analysis (LDA) and reduction on the data set obtained by exposing a **[PMAA-*co*-AAPBA•HT]** complex to four mono- and four disaccharides in neutral buffered water (50 mM HEPES at pH 7.4).

Table S3 LDA loading table for the first three factors from the analysis of data collected for the discrimination of small sugars using the [**PMAA-*co*-AAPBA•HT**] complex only.

Receptor	Type	Wavelengths	F1	F2	F3
[HT • PMAA-*co*-AAPBA]	absorbance	556 nm	1	7	21
		560 nm	2	14	0
		485 nm	4	1	5
		570 nm	13	53	48
		430 nm	0	3	1
		380 nm	28	14	15
		450 nm	5	0	1
	fluo	516/580 nm	0	1	0
		560/645 nm	0	1	0
		380/580 nm	47	6	9
		485/645 nm	0	0	0

HT = partially oxidized, ascorbate stabilized hematoxylin / hematein dye.

Factor 1 (F1) is mainly influenced by the fluorescence emission intensity at 580 nm and the absorbance at 380 nm.

Factor 2 (F2) mostly reflects effects of absorption at 570 nm, 560 nm and 380 nm.

Spontaneous oxidation of hematoxylin in air

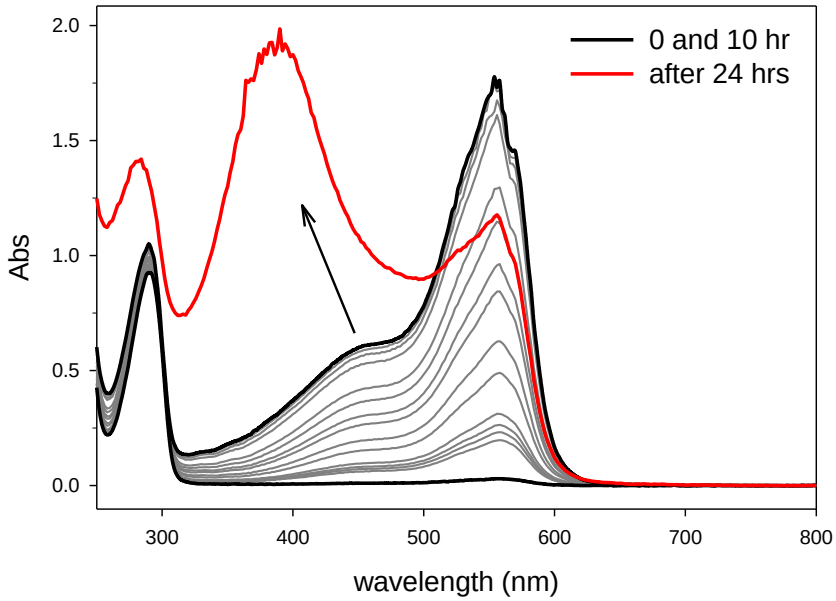


Figure S5 Oxidation of hematoxylin (HT) by atmospheric O_2 in buffered water (50 mM HEPES buffer) at pH 7.4 and room temperature: black: evolution in the first 10 hours of the "ripening" process; red: spectrum after 24 hrs. [**HT**] = 100 μM

Hematoxylin spontaneously oxidized in solution the presence of atmospheric oxygen. The process is well-known to histologists and cell biologists, who use a "ripened" (i.e. fully oxidized) HT solution for tissue staining. The spectra in the figure above show the spectral changes associated with the oxidation process as function of time. Below are kinetic profiles for the same oxidation process obtained in the same conditions, but at different starting concentrations of hematoxylin.

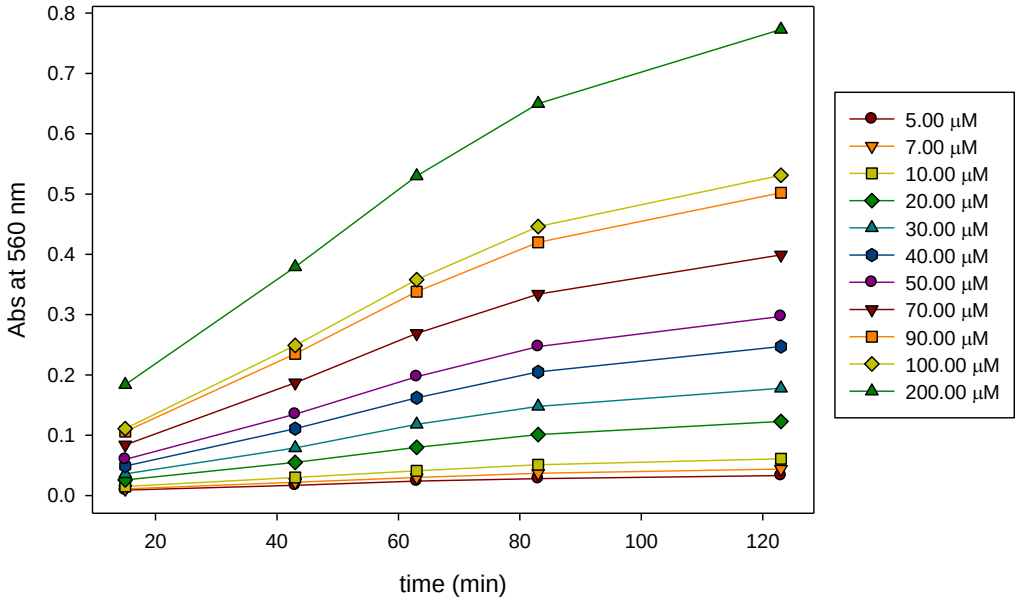


Figure S6 The time evolution of absorption at 560 nm for solutions of **HT** at different initial concentrations, as the dye is oxidized by atmospheric O_2 in buffered H_2O (pH 7.4) at room temperature.

Stability of partially oxidized HT solutions in the presence of ascorbate

The following are spectra and the corresponding time-dependent profile for repeated measurements of a stabilized solution of partially oxidized **HT** dye in the presence of two equivalents of ascorbate. It is clear from the data that these stabilized solutions retain the same properties for long periods. All experiments reported in this work were carried out with freshly oxidized and stabilized solutions of **HT**, and were completed well within the first two hours of preparation, in which period the solution's optical properties and composition were essentially unchanged, as shown below.

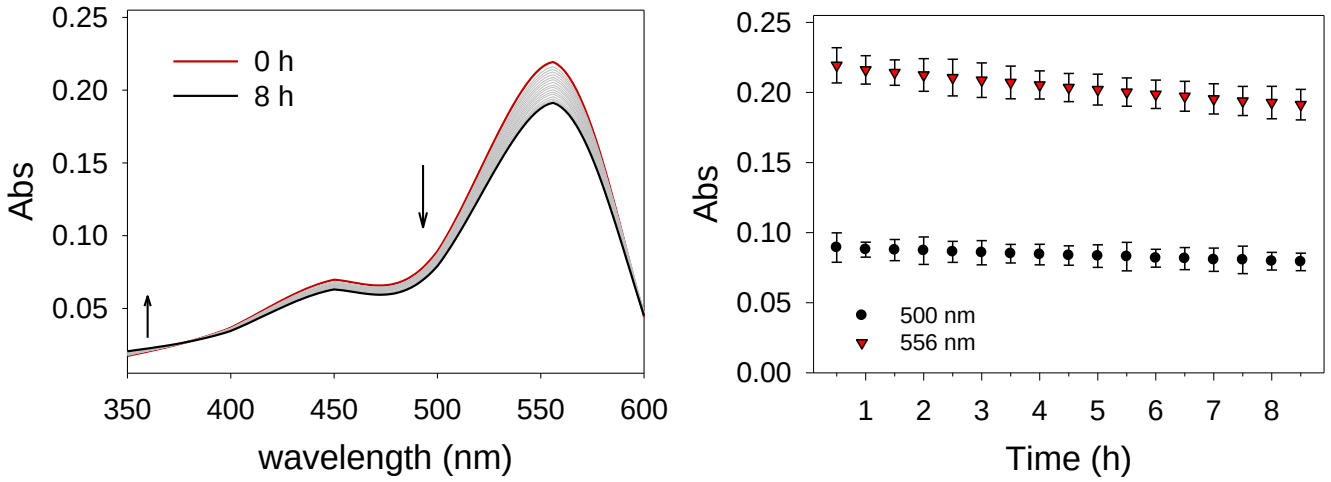


Figure S7 Stability of partially oxidized and stabilized solutions of hematoxylin (**HT)** dye. Left: family of spectra recorded over 8 hours. Right: time-dependent profiles obtained from these spectra. [**HT**] = 100 μM, [ascorbate] = 200 μM in buffered water (50 mM HEPES buffer) at pH 7.4 and at 25°C.

The spectra were obtained from a solution prepared as follows: an **HT** solution (100 μM in neutral buffered water) was left to oxidize in the presence of atmospheric O_2 for 30 min. After that time, two equivalents of ascorbate were added (using a solution obtained by dissolving ascorbic acid in the same buffer, whose pH was adjusted to 7.4). The solution was then dispensed on 96-well plates and measurements were carried out on a multiwell plate reader, whose sample compartment was electrically thermostatted at 25°C.

Composition of partially-oxidized dye solutions

Using data from the atmospheric oxidation profiles reported above (**Figure S5**), as well as spectroscopic data on the stability of the HT + ascorbate solutions (**Figure S7**), we were able to calculate the relative percentages of oxidized (= hematein) and reduced (= hematoxylin) forms of the dye in the partially oxidized and ascorbate-stabilized solution.

$$\%\ \text{oxidized} = \frac{\text{Abs}(\text{partially oxidized solution}) - \text{Abs}(\mathbf{HT}\ \text{alone}, t = 0)}{\text{Abs}(\text{fully oxidized}\ \mathbf{HT}, t = 8\ \text{hrs}) - \text{Abs}(\mathbf{HT}\ \text{alone}, t = 0)}$$

Using absorbance data at 556 nm, which remains well within the linearity range of the spectrophotometer in use, the values required in the formula above were reported in the following table.

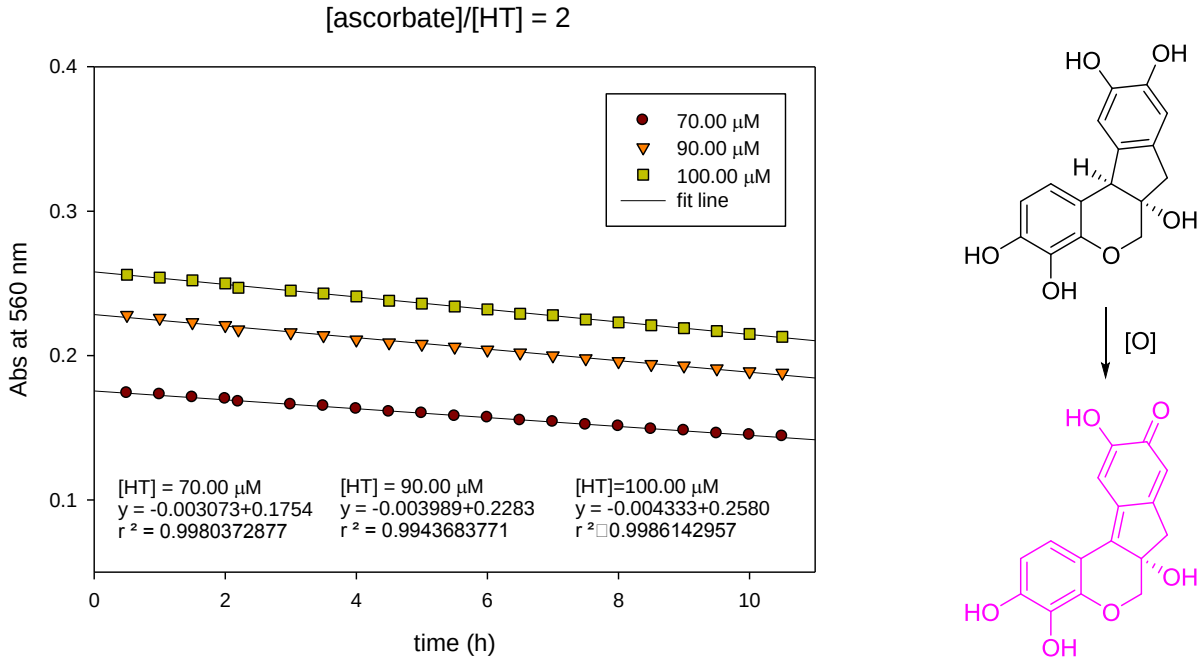
Table S4 Absorbance values at 556 nm used in the calculation of the composition of the partially oxidized, stabilized **HT** solutions. [**HT**] = 100 μM, [ascorbate] = 200 μM in buffered water (50 mM HEPES buffer) at pH 7.4 and at 25°C

Abs	partially oxidized solution	0.2194
Abs	**HT** alone, *t* = 0	0.0412
Abs	**HT** alone, *t* = 8 hrs	1.5053

$$\%\ \text{oxidized} = \frac{0.2194 - 0.0412}{1.5053 - 0.0412} \times 100 = 12\%$$

According to these calculations, the composition of the working solutions was 12% oxidized (hematein) form + 88% reduced (hematoxylin) form.

Ascorbate-stabilized, oxidized hematoxylin (HT) solution



Scheme S1 (Left) Ascorbate stabilizes the absorption of oxidized **HT** dye solution in pH 7.4 HEPES aqueous buffer over 10 hours. (Right) Reduced (hematoxylin) and oxidized (hematein) forms of the **HT** dye.

We found conditions to oxidize hematoxylin in air until the absorption of the solution reached an optimal level for our experiments (between 0.3 and 0.5 a.u.), then added two equivalents of ascorbate to prevent further oxidation and stabilize the solution's optical properties. As shown in Scheme S1, partially oxidized **HT** solutions at different concentrations could all be stabilized for up to 10 hours.

PMAA-*co*-AAPBA binding to cyanidin chloride (CC)

As aliquots of **PMAA-*co*-AAPBA** were added to a solution of cyanidin chloride dye (**CC**), absorption at 570 nm decreased (**Figure S3**), indicating complete binding of the dye present in solution.

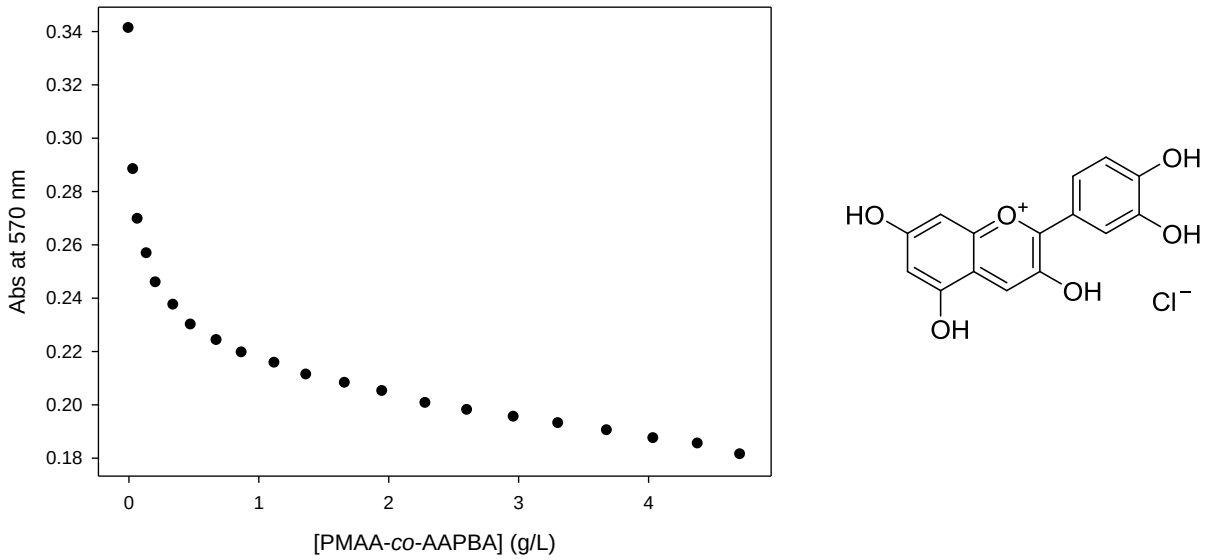


Figure S8 As aliquots of **PMAA-*co*-AAPBA** were added to the **CC** solution, absorption at 570 nm decreased. Experimental conditions: [cyanidin chloride] = 24.73 μM, T = 25ºC, pH 7.4 HEPES (50 mM) aqueous buffer.

No binding between PMAA-*co*-AAPBA and quercetin (QC)

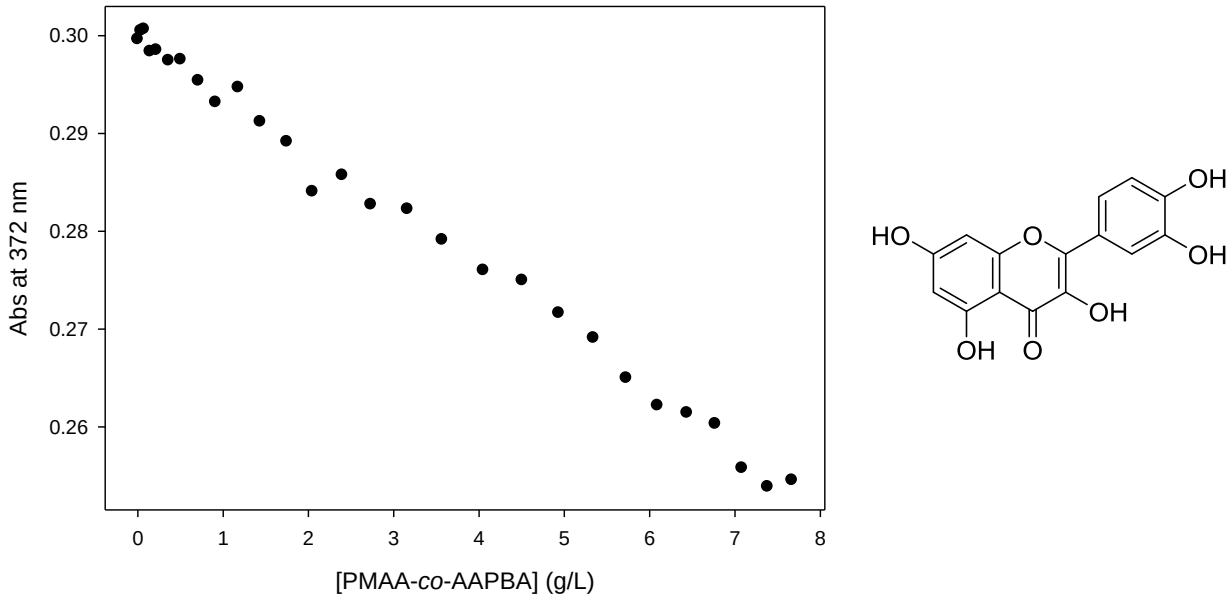


Figure S9 As aliquots of **PMAA-*co*-AAPBA** were added to the **QC** solution, absorption at 372 nm decreased linearly indicating that the addition of **PMAA-*co*-AAPBA** solution only diluted the **QC** solution in the cuvette. No binding between **PMAA-*co*-AAPBA** and **QC** was therefore observed. Experimental conditions: [quercetin] = 15.71 μM, T = 25°C, pH 7.4 HEPES (50 mM) aqueous buffer.