Supporting Information

Colorimetric pH indicator and boronic acid ensemble array for quantitative sugar analysis

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Experimental Section

Contents:

1. Chemicals and reagent

The following chemicals and reagents were purchased from Sigma Aldrich: 2-biphenylboronic acid, 2-fluoro-6-methoxyphenylboronic acid, 2-(hydroxymethyl)phenylboronic acid cyclic monoester, 3-nitrophenylboronic acid, phenylboronic acid, boric acid, phosphoric acid sodium monobasic, sodium hydroxide pellets (NaOH, 99.99% purity), D-(+)-glucose min 99.5%, D-fructose 99+% and sucrose. 4-methoxybenzeneboronic acid and 4-methylbenzeneboronic acid were purchased from Alfa Aesar. The spectroscopy grade of dimethyl sulfoxide (DMSO, 99.9% purity) was purchased from Acros. De-ionized water was prepared using the Picosystem (filtering system) from Hydro service and supplied company. Phosphate buffer (50 mM, pH 7.5) was prepared using phosphoric acid by titrating with NaOH solution. pH indicators were purchased from Chem Service, Sigma, Fluka, Kodak, Janssen and Aldrich. The saline solution was collected from the National University Hospital Pharmacy in Singapore. (Contents: NaCl -77.0mM, glucose -277.5mM)

2. Instruments and computer software

Polystyrene 384-well plates (clear flat bottom) were purchased from Greiner. All UV-VIS spectrum data were recorded from 350 nm to 750 nm using plate reader (Molecular Device; Spectra Max Plus 384). Principal component analyses were performed using Pipeline Pilot Student Edition v6.1 and R 2.3.1, and principal component analyses graphs were visually obtained using Miner 3D Professional.

3. Preparation of standard solutions and mixture samples

The pH indicator solutions (10 ~ 100mM) were prepared by measuring appropriate amounts (in grams) of the solid forms of the solutions and then dissolving in DMSO. The DMSO solution was then diluted in de-ionized water. The list of the dye solutions and their respective concentrations can be found in Table S1. Phosphate buffer solution (50 mM, pH 7.5) was prepared using phosphoric acid by titrating with NaOH solution. The solutions of the boronic and boric acids were prepared in the above phosphate buffer solution. Solutions of the carbohydrates were prepared in de-ionized water. For quantitative saline test, 1x saline solution was first neutralised with NaOH. Then, the solutions were diluted 4 times and 10 times using deionised water.

4. Array preparation and data acquisition

One dye and one boronic acid pair are considered as an ensemble probe. First, each solution of a pH indicator (25 μL) and boronic acid (25 μL) were prepared at corresponding concentration and place in 384 plate. Then, 2X sugar solutions (20 mM ~ 800 mM, 50 μL) were added 10 min before absorbance measurement. The absorbance spectra between 350 nm and 750 nm were recorded by the plate reader, at 10 nm interval. In order to evaluate the reproducibility of data, same experiments were repeated on another day. The absorbance spectra of the plate were then measured and the fold change value calculated using the previously reported procedure[1].
If the intensity of the analyte test is I_0 and that of the control is I_0, the fold-change, F, can be expressed as follows [Eq. (1)]:

$$F = \frac{I'_0}{I_0}$$ …… Eq. (1)

For the second class of dyes, there are two absorbance max peaks: one for control (λ_1) and a new peak that appeared in the test solution (λ_2). The effective fold-change can be calculated as follows [Eq. (2)]:

$$F = \left(\frac{I_1}{I_2}\right)\left(\frac{I'_2}{I'_1}\right)$$ …… Eq. (2)

Table S1. Absorbance maximum wavelengths of pH indicators and assay conditions. Detail data acquisition and process protocols were previously reported.[2]

ID of pH indicator	Dye name	λ_{max} (nm)	λ_1 (nm)	λ_2 (nm)	Concentration (mM)	DMSO (%)
1	Methyl orange		460	510	0.07	0.70
2	Alizarin yellow R		370	420	0.50	0.50
3	Eriochrome blue black R		550	510	0.30	3.00
4	Aniline blue black	620			0.04	0.40
5	Congo red		500	570	0.07	0.70
6	Evans blue	590			0.07	0.70
7	Brilliant yellow		400	500	0.05	0.50
8	Dibromofluorescein	500			0.02	0.2
9	Phloxine B	540			0.03	0.3
10	Neutral red		530	450	0.07	0.7
11	Alizarin		430	530	0.42	4.20
12	Alizarin red S (ARS)		420	520	0.42	4.20
13	Amaranth (acid red 27)		520	460	0.25	2.50
14	Trypan blue	590			0.05	0.5
15	Biebrich scarlet	510			0.12	1.20
16	Ponceau S	520			0.05	0.50
17	Victoria Blue B	610			0.05	0.50
18	Eosin bluish	520			0.03	0.30
19	Bromophenol blue		590	440	0.05	0.50
20	Chlorophenol red		430	570	0.08	0.40
21	Rose Bengal	550			0.05	0.50
22	Thymol blue		430	600	0.22	2.20
23	Ethyl violet	590			0.02	0.2
24	Cresol red		430	600	0.22	2.20
25	Metanil yellow	430			0.12	1.20
26	4-Phenylazoaniline		370	490	0.13	1.30
27	Erythrosin B		530	550	0.01	0.10
28	Ethyl orange		470	510	0.12	1.20
29	Bromocresol green		620	440	0.17	1.70
30	Resazurin		600	530	0.12	1.20
31	Nitrazine Yellow		460	590	0.06	0.60
32	Bromothymol Blue		430	620	0.15	1.50
33	m-Cresol Purple		430	570	0.25	2.50
34	Pyrocatechol violet		440	610	0.20	2.00
35	Xylenol orange		430	570	0.15	1.50
36	2',7'-Dichlorofluorescein		500	490	0.05	0.50
37	Bromochlorophenol Blue		590	440	0.03	0.30
38	Quinaldine red	500			0.07	0.70
39	Methyl red		520	430	0.02	0.20

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Table S2. Selected pH indicator and boronic acid pairs.

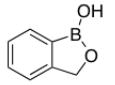
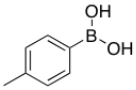
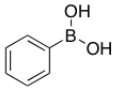
	pH indicator #1 **Methyl orange**	pH indicator #10 **Neutral Red**	pH indicator #11 **Alizarin**	pH indicator #12 **Alizarin Red S**	pH indicator #22 **Thymol Blue**	pH indicator #33 **m-Cresol Purple**
	EP-6		EP-11	EP-2		
	EP-7	EP-10				
				EP-1	EP-8	EP-4
				EP-3	EP-9	EP-5

Fig S1. Representative dose response evaluation for probe selection. 2-(hydroxymethyl)phenylboronic acid were mixed with (a) aniline blue black (b) alizarin red S (c) Ethyl orange.

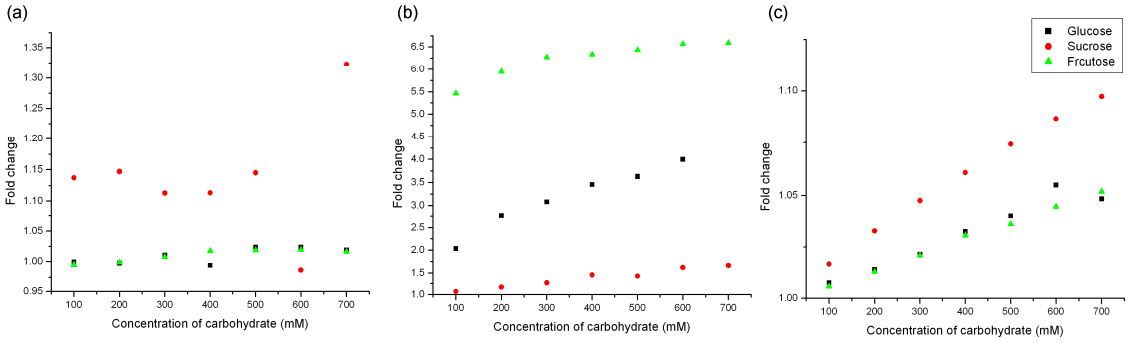
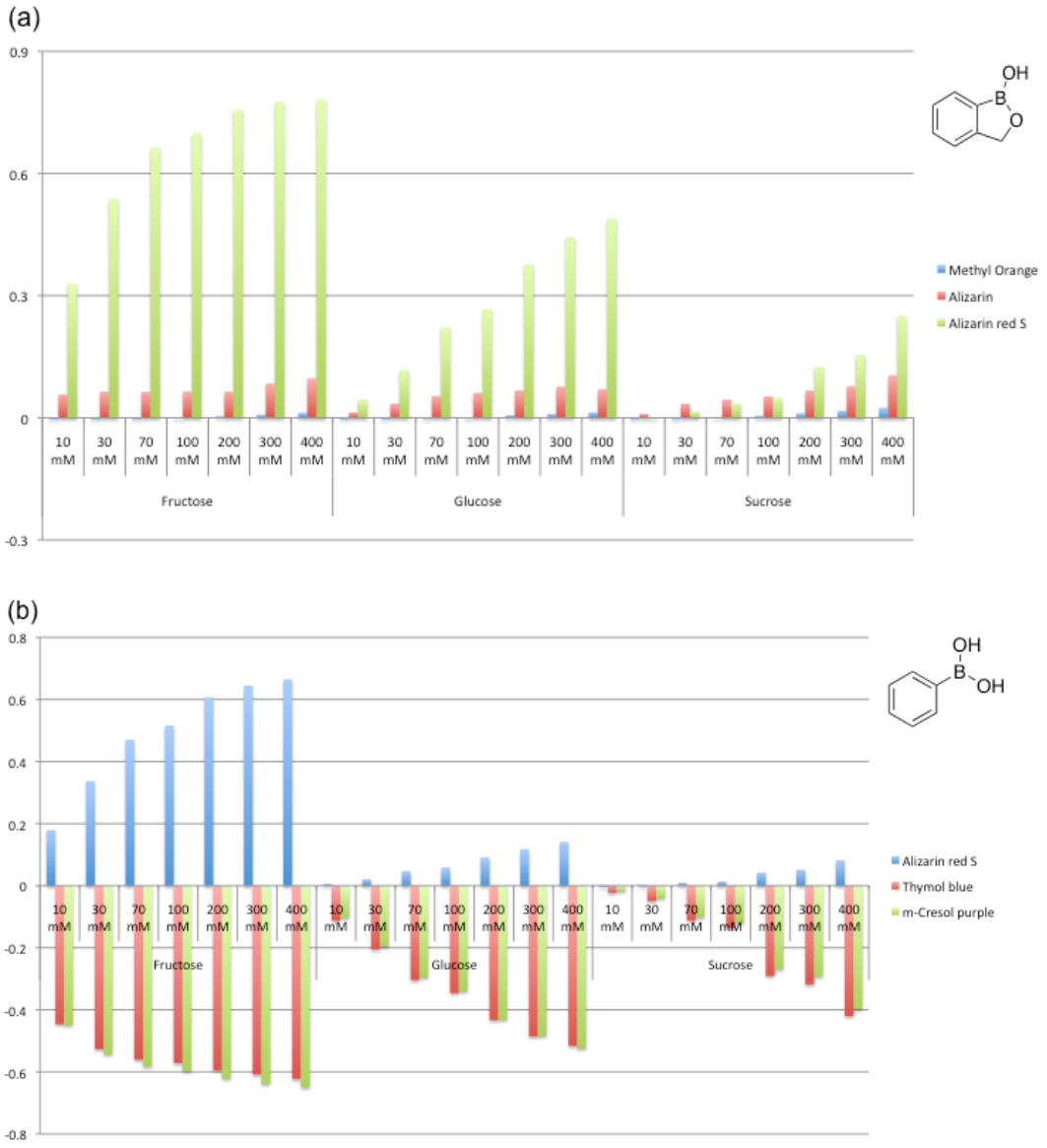
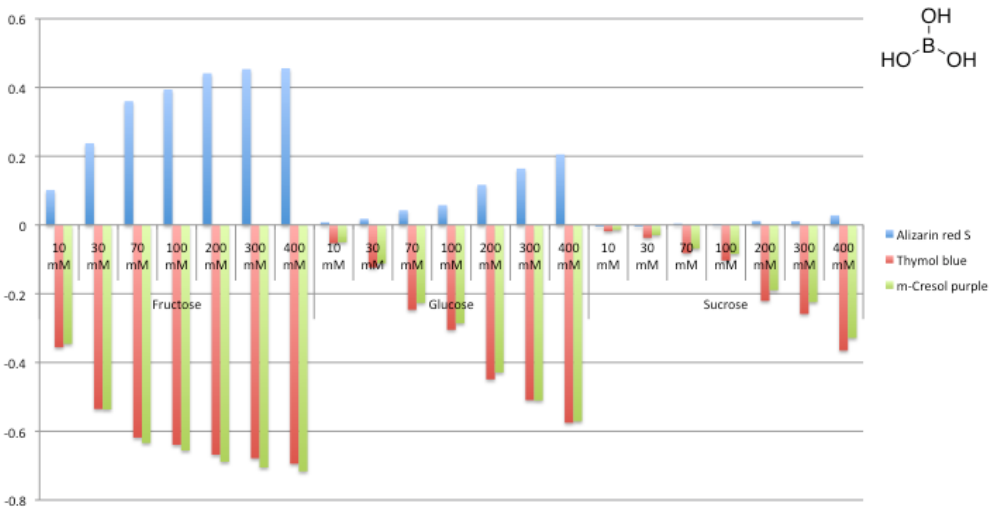


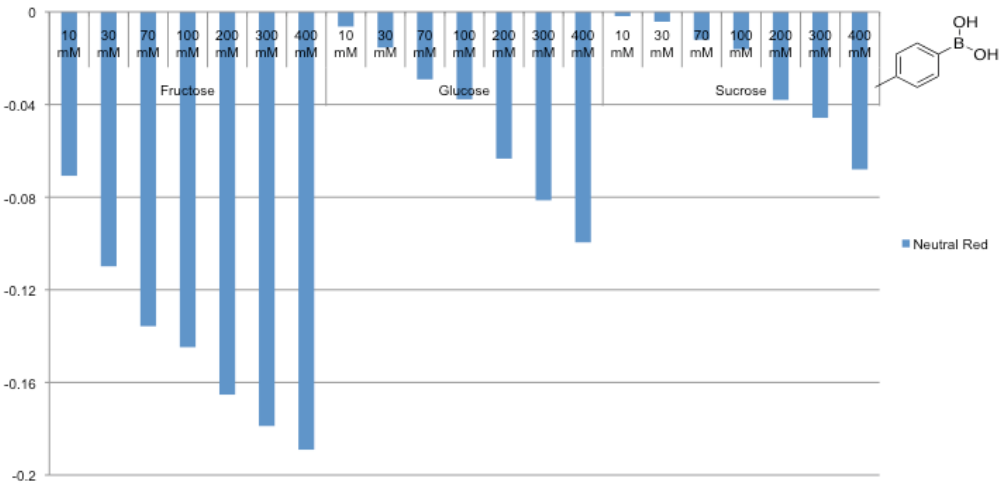
Fig S2. Bar graph of ensemble array's Log(fold) values for three cabohydrates (glucose, fructose, sucrose). Ensemble probes with (a) 2-(hydroxymethyl)phenylboronic acid, (b) phenyl boronic acid, (c) boric acid, (d) 4-methylphenyl boronic acid.



(c)


0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
10 mM
30 mM
70 mM
100 mM
200 mM
300 mM
400 mM
Fructose
Glucose
Sucrose
Alizarin red S
Thymol blue
m-Cresol purple
OH
HO
B
OH

(d)


0
-0.04
-0.08
-0.12
-0.16
-0.2
10 mM
30 mM
70 mM
100 mM
200 mM
300 mM
400 mM
Fructose
Glucose
Sucrose
Neutral Red
OH
B
OH

(e)

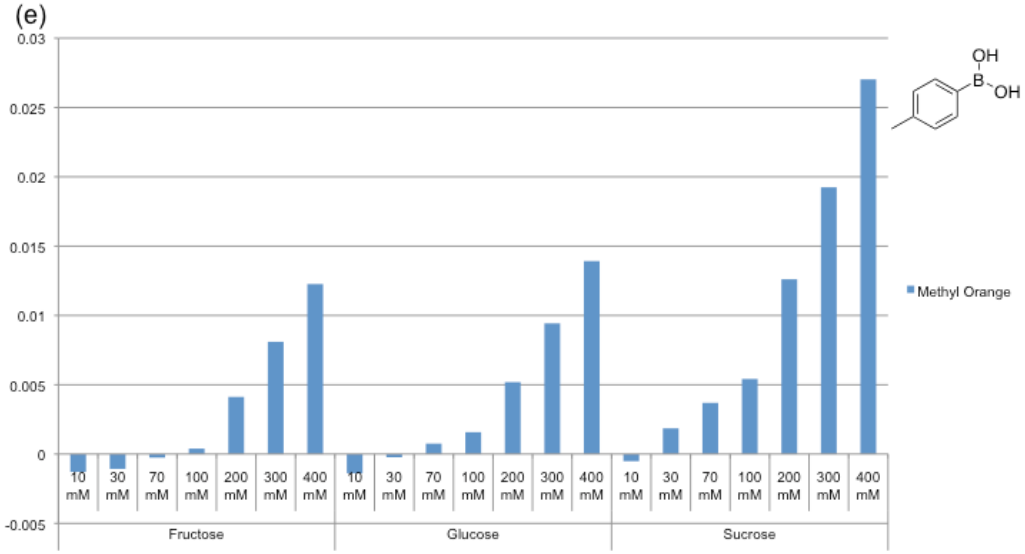
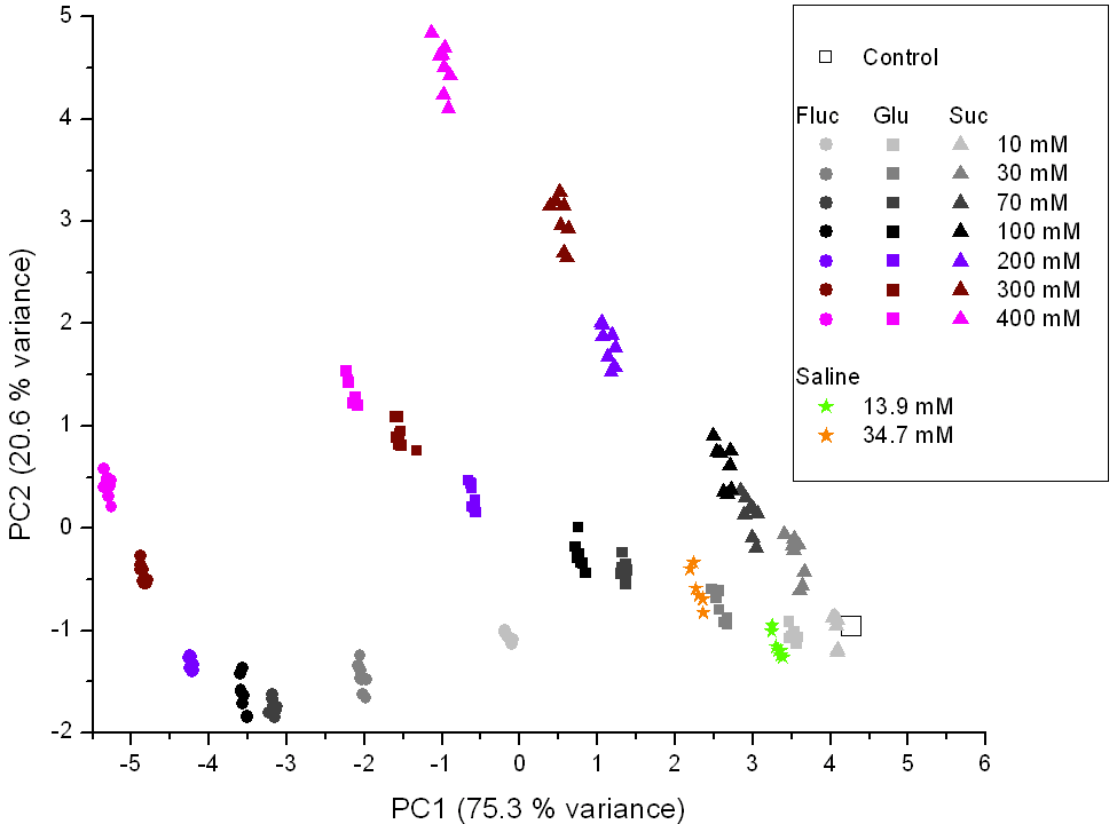

0.03
0.025
0.02
0.015
0.01
0.005
0
-0.005
10 mM
30 mM
70 mM
100 mM
200 mM
300 mM
400 mM
Fructose
Glucose
Sucrose
Methyl Orange
OH
B
OH

Fig S3. Principal component analysis (PCA) plot for real-mixture sample, saline. 11 selected ensemble probes were used to generate PCA plot (Table S2). Fruc: fructose, Glu: glucose, Suc: sucrose



References:

1. J. W. Lee, J. S. Lee and Y. T. Chang, *Angew. Chem. Int. Ed.* 2006**, 45**, 6485
2. J. W. Lee, J. S. Lee, M. Kang, A. I. Su and Y. T. Chang, *Chem-Eur J*, 2006, **12**, 5691.