Electronic Supplementary Material (ESI) for New Journal of Chemistry
This journal is © The Royal Society of Chemistry and The Centre National de la Recherche Scientifique 2013

Electronic Supplementary Information

The utilization of pH sensitive spirocyclic rhodamine dyes for monitoring D-fructose consumption during a fermentation process

Erman Karakuş, Muhammed Üçüncü, Ritchie Eanes and Mustafa Emrullahoğlu*[a]

[a] Department of Chemistry, Faculty of Science, İzmir Institute of Technology, Urla-35430, İzmir, Turkey

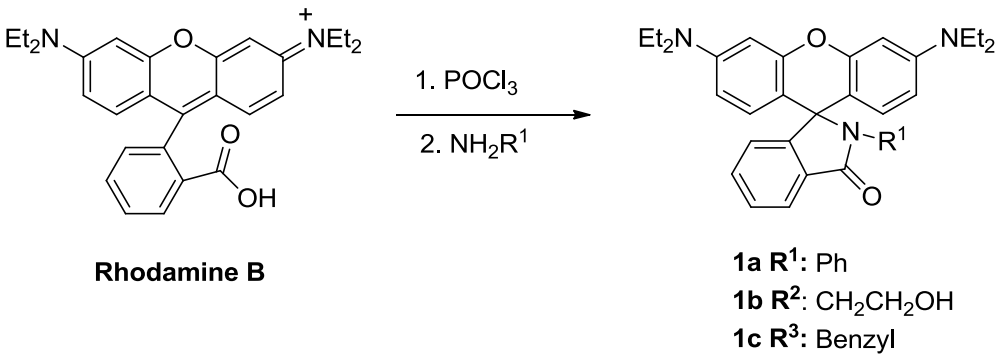
CONTENTS **PAGE**

This journal is © The Royal Society of Chemistry and The Centre National de la Recherche Scientifique 2013

1. General Methods

All reagents for synthesis were purchased from commercial suppliers (Aldrich an Merck) and used without further purification. Absolute methanol and distilled water were used throughout the experiment. The pH was recorded by WTW InoLab pH 720 precision pH meter (Weilheim, Germany). UV absorption spectra were obtained on a Shimadzu UV-2550 Spectrophotometer. Fluorescence emission spectra were obtained using a Varian Cary Eclipse Fluorescence spectrophotometer. The slit width was 5.0 nm for both excitation and emission. The emission spectra were integrated over the range 525 nm to 700 nm in 2.00 mm path length quartz cells (0.5 mL volume). All measurements were conducted at least in triplicate.

2. Synthesis of 1a, 1b and 1c from Rhodamine B



Rhodamine B

1a R^1: Ph
1b R^2: CH_2CH_2OH
1c R^3: Benzyl

Scheme 1. Synthesis of Rhodamine derivatives **1a-c**

The product **1a**[1], **1b**[2] and **1c**[3] were synthesized according to the literature procedures.

References

1. X. Zhang, Y. Shiraishi and T. Hirai, *Tetrahedron Lett.*, 2007, **48**, 5455-5459.

2. Y. Zhao, X.-B. Zhang, Z.-X. Han, L. Qiao, C.-Y. Li, L.-X. Jian, G.-L. Shen, R.-Q. Yu, *Anal. Chem.*, 2009, **81**, 7022-7030.

3. A. Chatterjee, M. Santra, N. Won, S. Kim, J. K. Kim, S. B.Kim, K. H. Ahn, *J. Am. Chem. Soc.*, 2009, **131**, 2040-2041.

This journal is © The Royal Society of Chemistry and The Centre National de la Recherche Scientifique 2013

3. UV-Vis Titration of SA-1 to SA-5 with Saccharide Derivatives

A solution of **1a, 1b** and **1c** (25 μM) and all arylboronic acid derivatives (50 mM) were prepared in distilled water and absolute methanol (3/7 v/v). Also, all saccharide solutions were prepared in distilled water and absolute methanol (3/7 v/v). A solution containing compound **1a** (or **1b** and **1c**), arylboronic acid and different portions of saccharide solutions (1, 5, 10, 25, 50, 100, 250, and 500mM) were mixed in falcon tubes. They were placed in a quartz cell (2.0 mm width), and the absorption spectrum was recorded at 25 ^{0}C and their corresponding UV-Vis curves were recorded.

4. Effect of Water Content

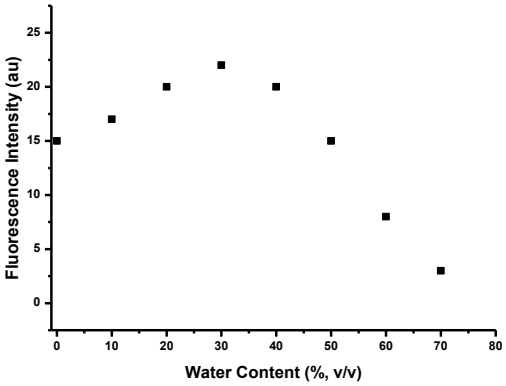


Figure S1. Effect of water content on the fluorescence intensity of **SS-1** (**1a** [25μM] and **PBA** [50mM]) upon addition of D-fructose [100mM]. λ_{ex}:525nm

5. Change in Acidity upon Addition of Increasing Concentrations of D-Fructose to SS-1

Table S1. Change in acidity upon addition of increasing concentrations of D-fructose to **SS-1** in water/methanol (3:7, v/v)

D-fructose, [mM]	0	1	5	10	25	50	100	250	500
pH [a]	5.90[a]	5.85	5.53	4.83	4.44	4.32	4.04	3.89	3.82
ΔpH [b]	0	0.05	0.37	1.07	1,46	1,58	1,86	2.01	2.08

[a] relative pH values of sensing system **SS-1** after addition of increasing concentrations of D-fructose [b] decrease in pH of the solution (**SS-1**) after addition of D-fructose determined by a standard by pH meter

This journal is © The Royal Society of Chemistry and The Centre National de la Recherche Scientifique 2013

6. pH Change Profile for SS-2 upon Addition of D-Fructose at Various pH Conditions

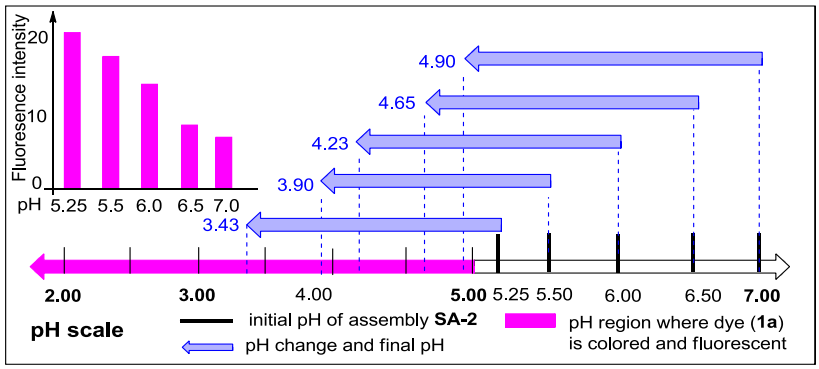
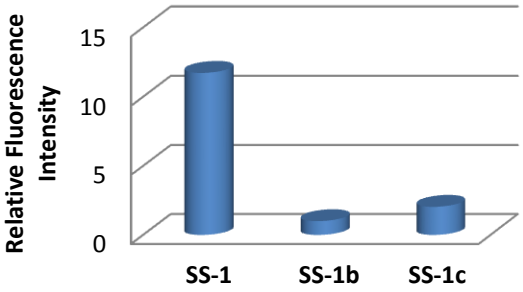


Figure S2. pH change profile for **SS-2** upon addition of D-fructose [100mM] at various pH conditions; the inset shows the relative fluorescence intensities of **SS-2** upon addition of D-fructose [100mM] at various pH conditions (pH 5.2, 5.5, 6.0, 6.5 and 7.0).

7. Emission Intensity of the Sensing System Employing Different Rhodamine Derivatives (1a, 1b and 1c)



SS	(boronic acid + dye)
SS-1	PBA + **1a**
SS-1b	PBA + **1b**
SS-1c	PBA + **1c**

Figure S3. Relative fluorescence intensity of sensing system employing different dyes (PBA [50mM] + dye **1a**, **1b**, **1c** [25 μM]), upon addition of D-fructose [100mM]. λ_{ex}:525nm

This journal is © The Royal Society of Chemistry and The Centre National de la Recherche Scientifique 2013

8. Absorption and Emission Spectra of SS-2 at Initial pH 7.0 and 5.2

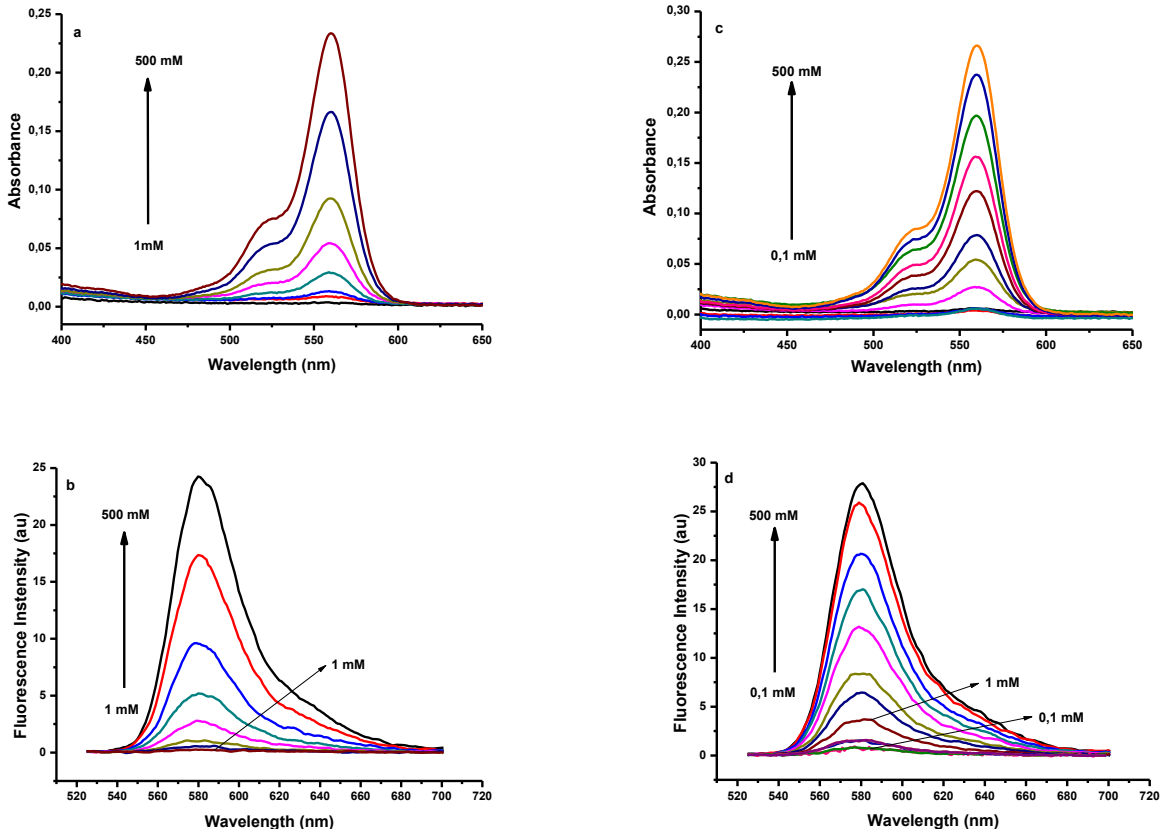


Figure S4. a) Absorption and b) Emission spectra of **SS-2** in water/methanol (3:7, v/v, **pH:7.0**) c) Absorption and d) emission spectra of **SS-2** in water/methanol (3:7, v/v, **pH 5.2**) with increasing concentrations of D-fructose [0.1, 0.25, 0.5, 0.75, 1.0, 5.0, 10, 25, 50, 100, 250 and 500 mM].

9. pH-Dependent Fluorescence Response of Rhodamine 1a

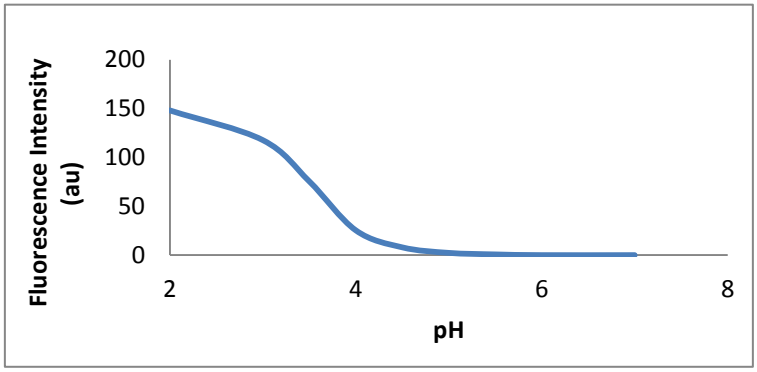
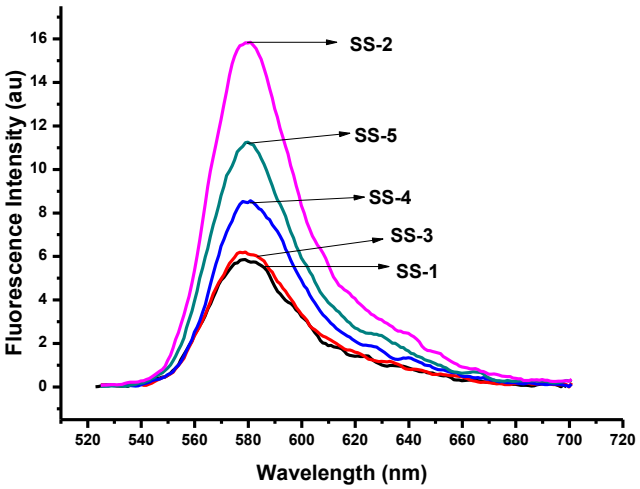


Figure S5. pH-Dependent fluorescence response of dye **1a** in water/methanol (3:7, v/v).

This journal is © The Royal Society of Chemistry and The Centre National de la Recherche Scientifique 2013

10. Fluorescence Intensity Spectrum upon Addition of D-Fructose to the Sensing Systems Using Variations of Aryl boronic Acids



System	arylboronic acid + **1a**
SS-1	PBA
SS-2	*p*-CF_3-PBA
SS-3	*p*-CH_3O-PBA
SS-4	*p*-CH_3- PBA
SS-5	*p*-Br-PBA

Figure S6. Emission spectra for **SS-1**, **SS-2**, **SS-3**, **SS-4** and **SS-5** in water/methanol (3:7, v/v) after the addition of D-fructose [100mM]. λ_{ex}:525 nm

11. Time Dependent Fluorescence Intensity Change

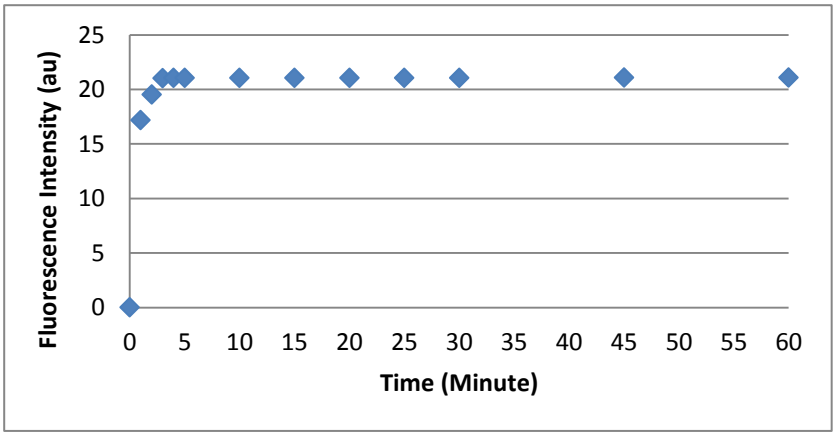


Figure S7. Time dependent fluorescence intensity change of **SS-2** upon addition of [100 mM] D-fructose in water/methanol (3:7, v/v, pH 5.2) λ_{ex}:525nm

This journal is © The Royal Society of Chemistry and The Centre National de la Recherche Scientifique 2013

12. Saccharide selectivity profiles of all sensing systems

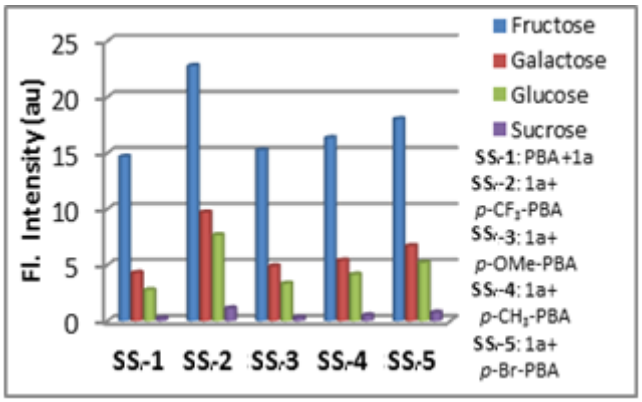


Figure S8. Fluorescence intensities for **SS-1, SS-2, SS-3, SS-4** and **SS-5** (dye/boronic acid (25μM/50mM)) after adding saccharides (D-fructose, D-glucose, D-galactose, and D-Sucrose, [100mM]) in water/methanol (3:7, v/v, pH 5.2). λ_{ex}:525nm

13. Colorimetric and Fluorometric Detection of D-fructose

Figure S9. Colorimetric response of **SS-2** (**1a** [25 μM] and *p*-CF_3-PBA [50 mM]) to various concentrations of D-fructose in water/methanol (3:7, v/v, pH 5.2). [left to right: 500, 250, 100, 50, 25, 10, 5, and 1 mM]

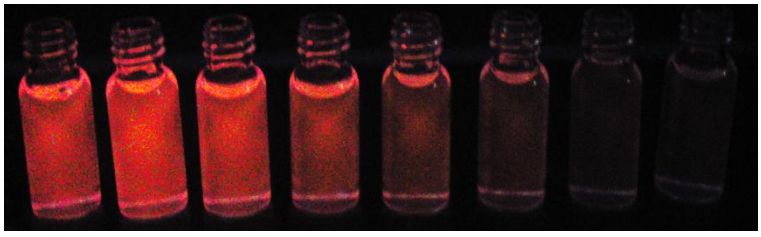


Figure S10. Fluorometric response of **SS-2** (**1a** [25 μM] and *p*-CF_3-PBA [50 mM]) to various concentrations D-frcutose in water/methanol (3:7, v/v, pH 5.2). [left to right: 500, 250, 100, 50, 25, 10, 5, and 1 mM]

This journal is © The Royal Society of Chemistry and The Centre National de la Recherche Scientifique 2013

14. Calibration Curve

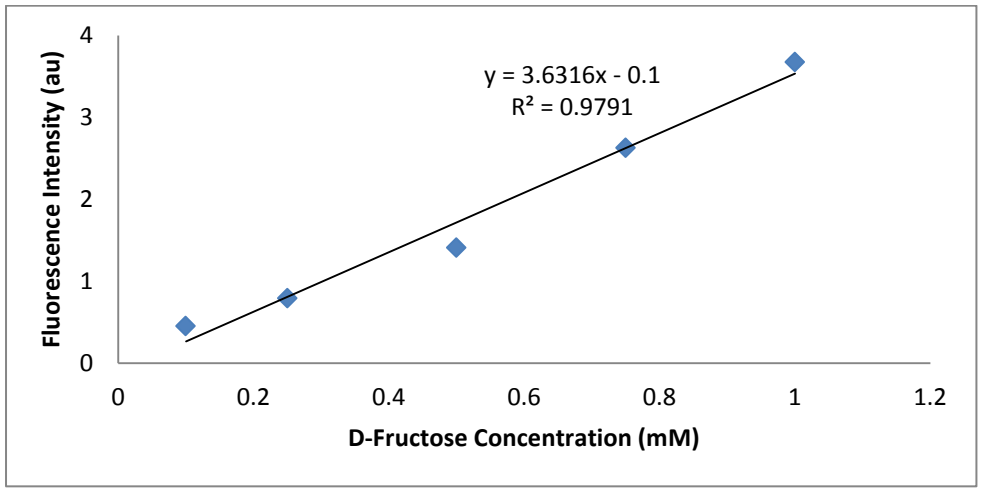


Figure S11. Calibration emission spectra of **SS-2** in water/methanol (3:7, v/v, **pH 5.2**) with increasing concentrations of D-fructose [0.1, 0.25, 0.5, 0.75, 1.0 mM]