Optical Glucose Detection Across the Visible Spectrum Using Anionic Fluorescent Dyes and a Viologen Quencher in a Two-Component Saccharide Sensing System

SUPPORTING INFORMATION

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Experimental Section.

General. The following fluorescent dyes were purchased from Molecular Probes or Aldrich and used as received:

Fluorescein-SA = fluorescein-5-(and-6-)sulfonic acid (MP#F1130);

Lucifer Yellow-I = lucifer yellow iodoacetamide (MP#L1338);

SR-B = sulforhodamine-B (Aldrich#23,016-2);

SR-101 = sulforhodamine-101 (Aldrich#28,491-2);

MPTS = methoxypyrenetrisulfonate (MP#335);

CTR = carboxytetramethylrhodamine (MP#C300);

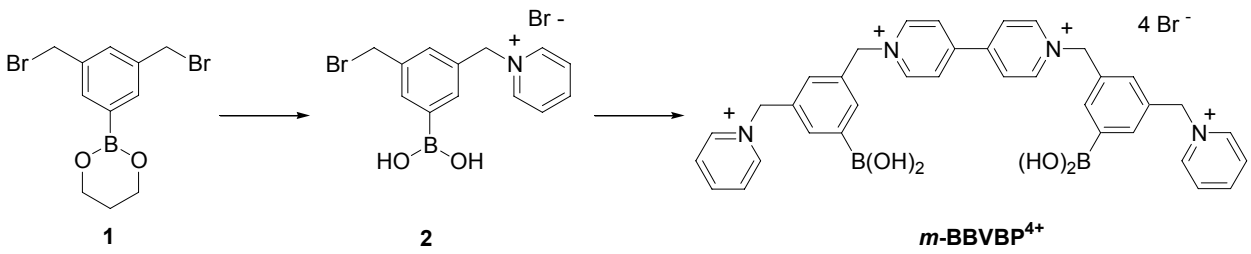
TSPP = tetrakis(4-sulfophenyl)porphine (MP#T6932);

TCPP = tetrakis(4-carboxyphenyl)porphine (MP#T6931)

The preparation of HPTS$(Lys)_3$ has already been described.[1] Perylenetetracarboxylic acid (PTCA) was prepared by simple alkaline hydrolysis of perylene dianhydride with

potassium hydroxide and used as the tetrapotassium carboxylate salt. All other reagents including HPTS dye were purchased from Aldrich Co. and used as received. All solvents were HPLC grade. DMF was stirred over CaH_2 for 2 days and filtered immediately before use. All reactions were conducted under an inert atmosphere of argon using air sensitive techniques. pH measurements were carried out on a Mettler Toledo MP 220 pH meter under argon. ^{11}B NMR spectra were measured on a Bruker 250 using BF_3:Et_2O as an external standard. ^{13}C and ^{1}H NMR spectra were measured on a Varian 500 referenced to TMS.

Synthesis of *m*-BBVBP^{4+} (4,4'-*N,N'*-bis-[benzyl-(3-methylene-pyridinium bromide)-5-(boronic acid)]-dipyridinium dibromide):



1 **2** ***m*-BBVBP^{4+}**

2-(3,5-Bis-bromomethyl-phenyl)-[1,3,2]dioxaborinane (1). To a 500-mL round bottom flask fitted with a condenser and a side-arm was added 3,5-dimethylphenylboronic acid (10.5 g, 70 mmol), calcium hydride (5.9 g, 140 mmol), and dichloroethane (300 mL). After 10 min of stirring under argon, 1,3-propane diol was added via syringe. The reaction was refluxed for 1.5 h, cooled to RT, and filtered. The clear filtrate was mixed with *N*-bromosuccinimide (27.4 g, 154 mmol) and 2,2'-azobisisobutryonitrile (2.3 g, 14 mmol), and refluxed for 3 h. The orange colored solution was cooled overnight, and the succinate crystals that formed were filtered off.

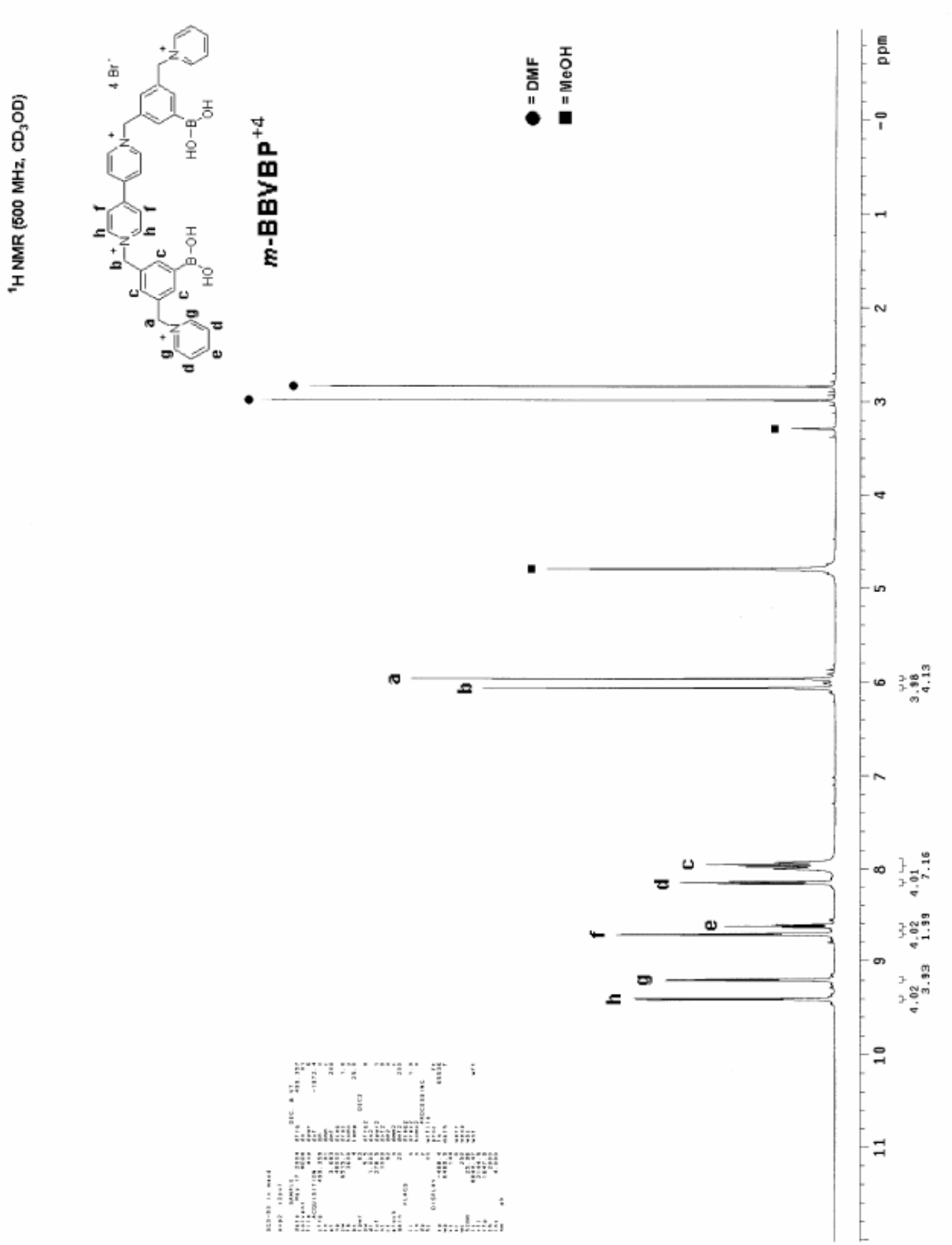
The filtrate was evaporated to dryness, leaving an off-white chunky solid, which was recrystallized from methanol (*ca.* 300 mL) to give 11.0 g (46 %) of pure **1**. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.07 (q, *J* = 5.5 Hz, 2H), 4.17 (t, *J* = 5.5 Hz, 4H), 4.49 (s, 4H), 7.48 (s, 1H), 7.74 (s, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 27.51, 33.39, 62.22, 131.92, 134.52, 137.73; ^{11}B NMR (80 MHz, $CDCl_3$) δ 28.5. Anal Calcd for $C_{11}H_{13}BBrO_2$: C, 37.98; H, 3.77; Br, 45.94. Found: C, 38.08; H, 3.68; Br, 46.12.

1-(3-Boronic acid-5-bromomethyl-benzyl)-pyridinium Bromide (2). Pyridine (0.56 mL, 7 mmol) was added via syringe to a solution of compound **1** (9.73 g, 28 mmol) in CH_2Cl_2 (370 mL) and CH_3OH (180 mL), and the reaction was stirred at 40 ^{o}C for 22 h. The CH_2Cl_2 was removed *in vacuo*, and the excess **1** which precipitated out of methanol was filtered off and washed with ice-cold methanol. The filtrate was concentrated down to *ca.* 20 mL, then acetone (*ca.* 300 mL) was added, followed by the addition of ether until turbidity occurred. Storage at – 4 ^{o}C for 24 h resulted in the formation of white needle-shaped crystals. The solid was collected by centrifugation, washed several times with acetone, and dried under argon to yield 1.3 g of pure 2 (48% yield). ^{1}H NMR (CD_3OD, 500 MHz) δ 4.57 (s, 2H), 5.88 (s, 2H), 7.64 (s, 1H), 7.75-7.90 (m, 2H), 8.13 (dd, *J* = 7.0, 7.5 Hz, 2H), 8.61 (tt, *J* = 8.0, 1.5 Hz, 1H), 9.10 (d, *J* = 5.5 Hz, 2H); ^{13}C NMR (CD_3OD, 125 MHz) δ 31.9, 64.1, 128.4, 130.9, 133.0, 133.8, 135.6, 139.1, 144.6, 146.1; ^{11}B NMR (80 MHz, CD_3OD) δ 28.3.

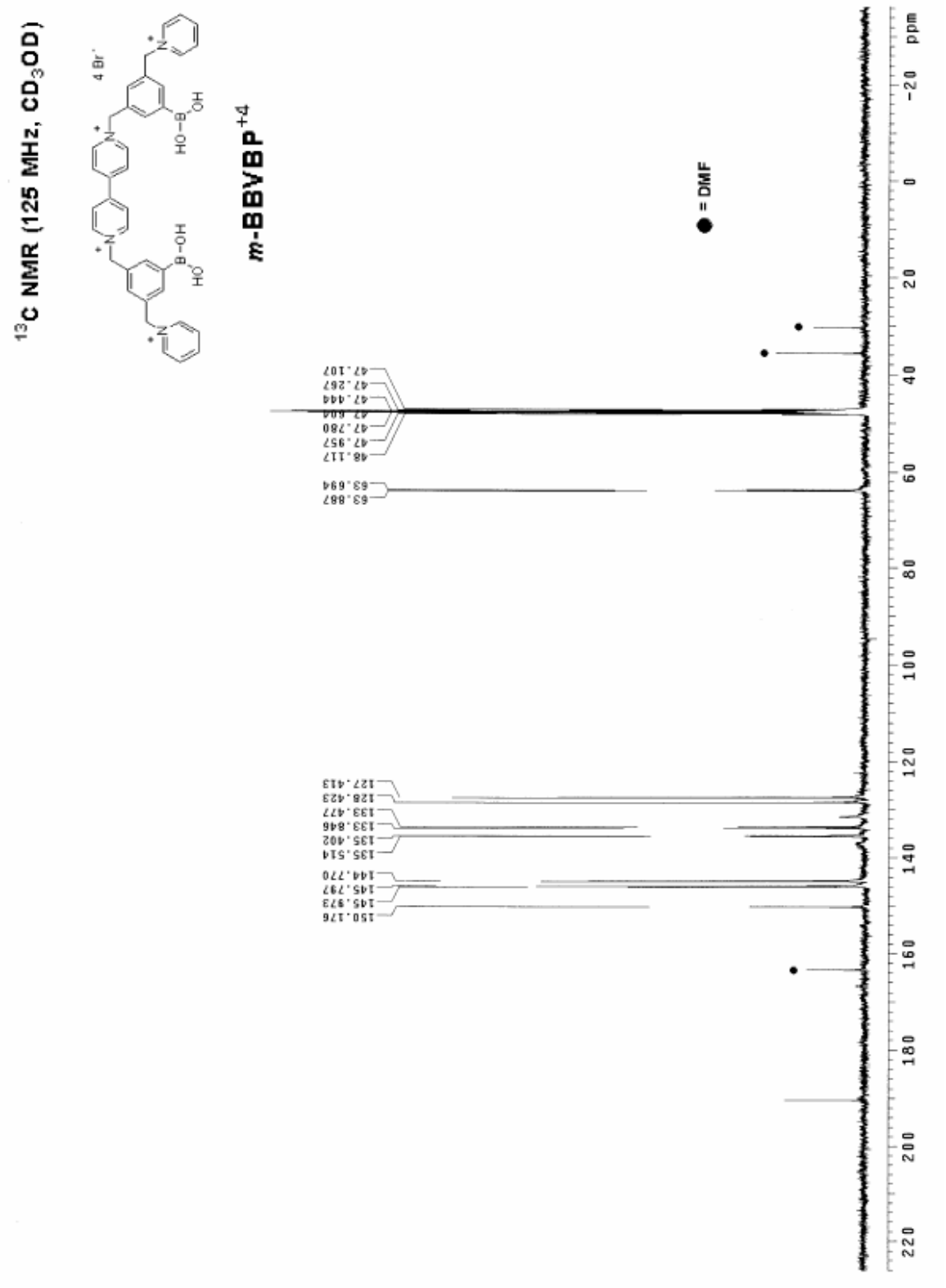
***m*-BBVBP^{4+} (4,4'-*N,N*'-bis-[benzyl-(3-methylene-pyridinium bromide)-5-(boronic acid)]-dipyridinium dibromide).** To a solution of **2** (0.49 g, 1.26 mmol) in DMF (20

mL) was added 4,4'-dipyridyl (0.094 g, 0.6 mmol), and the reaction was stirred at 70 ^{o}C for 72 h. The bright yellow precipitate was collected by centrifugation, washed with DMF, then acetone, and dried under a stream of argon to yield pure **7** (0.28 g, 51% yield). ^{1}H NMR (CD_3OD, 500 MHz) δ 5.97 (s, 4H), 6.07 (s, 4H), 7.93-7.99 (m, 6H), 8.15 (t, *J* = 7.0 Hz, 4H), 8.62 (tt, *J* = 7.5, 1.5 Hz, 2H), 8.71 (d, *J* = 7.0 Hz, 4H), 9.19 (d, *J* = 5.5 Hz, 4H), 9.40 (d, *J* = 7.0 Hz, 4H); ^{13}C NMR (CD_3OD, 125 MHz) δ 63.7, 63.9, 127.4, 128.4, 131.5, 133.5, 133.8, 135.4, 135.5, 144.8, 145.8, 146.0, 150.2; ^{11}B NMR (80 MHz, CD_3OD) δ 25.9.

^{1}H NMR of *m*-BBVBP^{4+}:



^{13}C NMR of *m*-BBVBP^{4+}:



Fluorescence Emission and UV-vis Absorption Studies (General). All studies were carried out in pH 7.4 buffer solution prepared with water purified via a Nanopure Ultrafiltration system. Buffer solution (pH 7.4, 0.1 ionic strength) was freshly prepared using KH_2PO_4 and Na_2HPO_4. Fluorescence spectra were taken on a Perkin-Elmer LS50-B luminescence spectrometer. The absorption spectra were taken on a Hewlett Packard 8452A Diode Array Spectrophotometer. All studies were carried out at 20 °C without exclusion of air. Excitation and Emission wavelengths for each dye are given in Figure 1 of the main paper. For fluorescence titration experiments, the added volume did not exceed 3 % of the total volume and the sample absorbance for fluorescent measurements was below 0.1.[2] All experiments except the quencher:dye optimization were carried out in triplicate and the error is reported as the standard deviation.

Data Analysis. All data was analyzed using the Solver (non-linear least-squares curve fitting) in Microsoft Excel. Regression statistics were determined using the SolvStat macro.[3]

Absorbance Studies to Show Complex Formation. Measurements were done *in situ* by taking the absorbance spectra of the dyes HPTS, HPTS$(Lys)_3$, PTCA, MPTS, and Fluorescein-SA at a series of quencher concentrations. The emission of each dye (2 mL of 1 x 10^{-5} M in buffer)* was first obtained, then aliquots of quencher (0.005, 0.05, 0.01, or 0.1 M) were added, the solution shaken for 60 sec, and the new absorbance was measured. In order to minimize the volume of quencher solution being added, more

highly concentrated quencher stock solutions were used for the titrations of dyes requiring high quencher concentrations to achieve significant perturbatrion in their absorbance spectra. *TCPP and TSPP were used at 4 x 10^{-6} M.

Absorbance Studies Calculations. Association constants (K_{UV}) were calculated by means of Benesi-Hildebrand plots and the expression:

$$b/(\Delta A) = 1/(S_t K_{UV} \Delta\epsilon[L]) + 1/(S_t \Delta\epsilon)$$

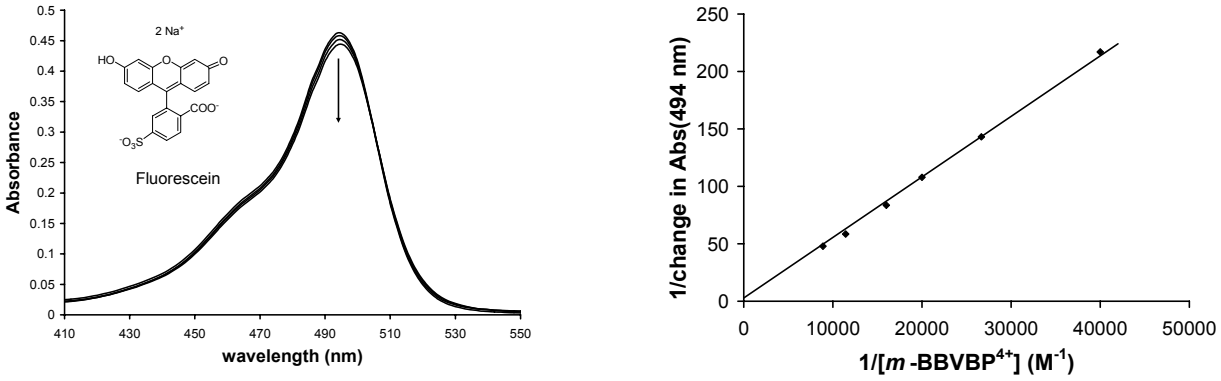
with association constants evaluated by,

$$K_{UV} = (y\text{-intercept})/(\text{slope}) = -(x\text{-intercept})$$

where b is the y-intercept, ΔA is the change in absorbance at the monitored wavelength, S_t is the substrate (dye) concentration, $\Delta\epsilon$ is the change in the molar absorptivity, and [L] is the ligand (quencher) concentration.[4]

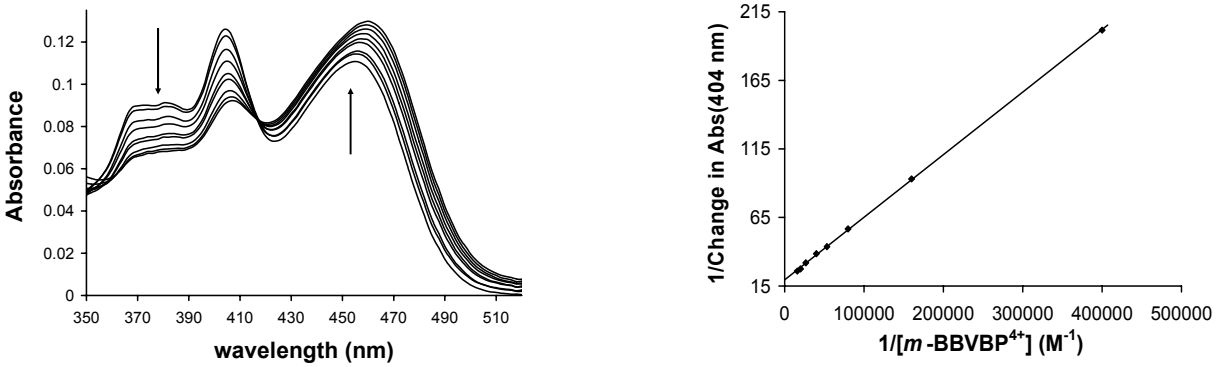
Absorbance:

Fluorescein-SA:



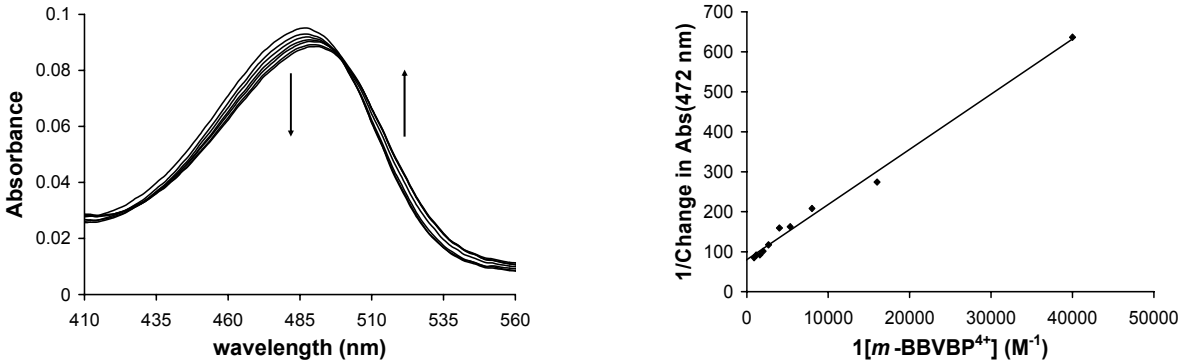
UV-Vis Absorbance Spectra and Benesi-Hildebrand Plot of Fluorescein-SA (1 x 10^{-5} M) in pH 7.4 buffer with increasing [*m*-$BBVBP^{4+}$].

HPTS:



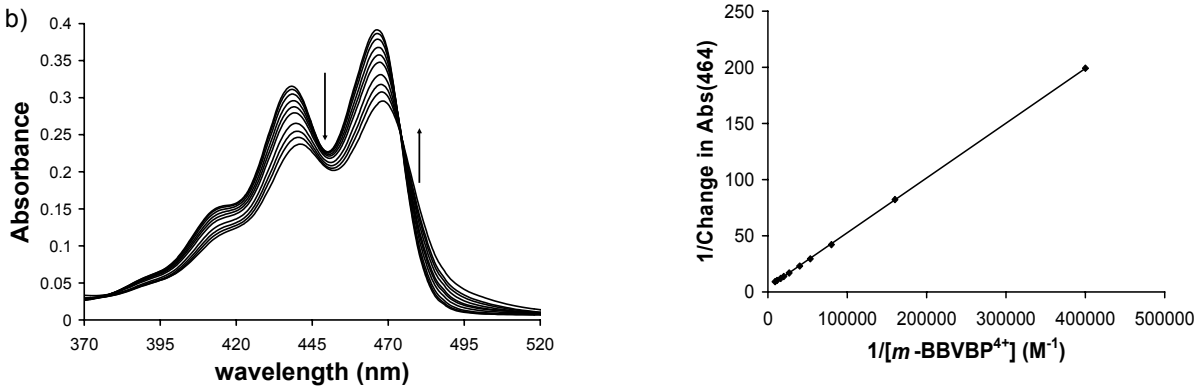
UV-Vis Absorbance Spectra and Benesi-Hildebrand plot of HPTS (1 x 10^{-5} M) in pH 7.4 buffer with increasing [*m*-$BBVBP^{4+}$].

$HPTS(Lys)_3$:



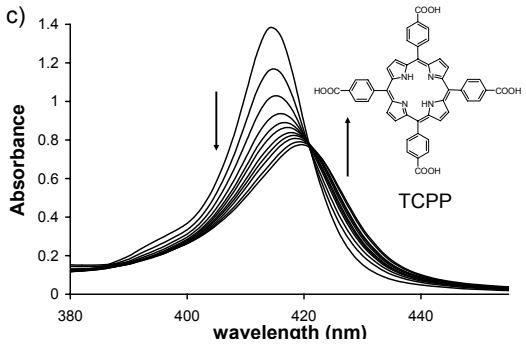
UV-Vis Absorbance Spectra and Benesi-Hildebrand plot of $HPTS(Lys)_3$ (1 x 10^{-5} M) in pH 7.4 buffer with increasing [*m*-$BBVBP^{4+}$].

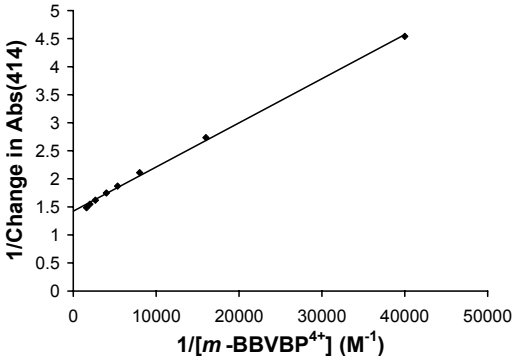
PTCA:



UV-Vis Absorbance Spectra and Benesi-Hildebrand plot of PTCA (1 x 10^{-5} M) in pH 7.4 buffer with increasing [*m*-$BBVBP^{4+}$].

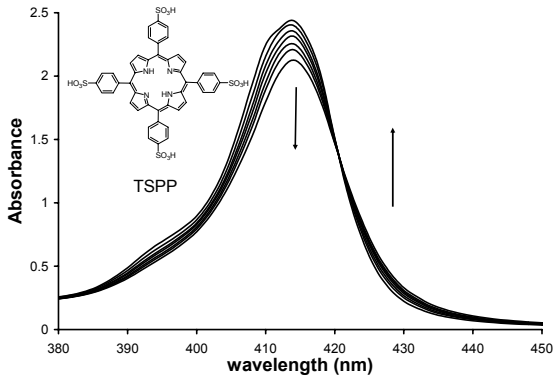
TCPP:

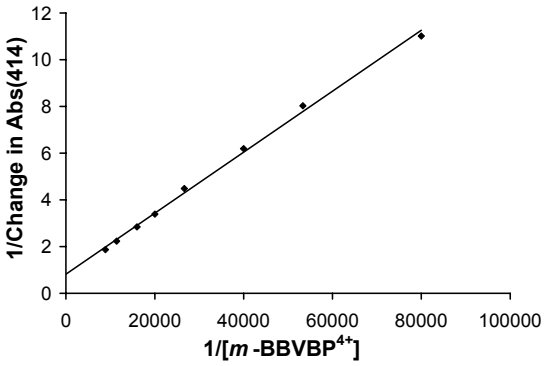




UV-Vis Absorbance Spectra and Benesi-Hildebrand plot of TCPP (4 x 10^{-6} M) in pH 7.4 buffer with increasing [*m*-$BBVBP^{4+}$].

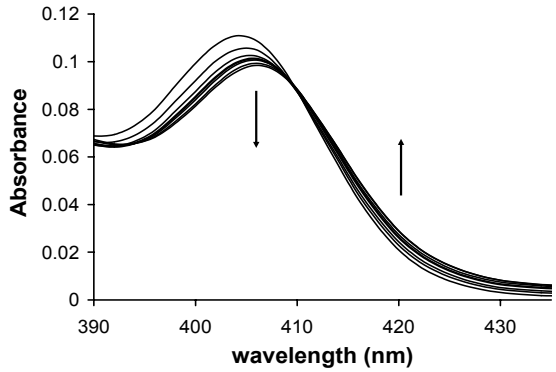
TSPP:

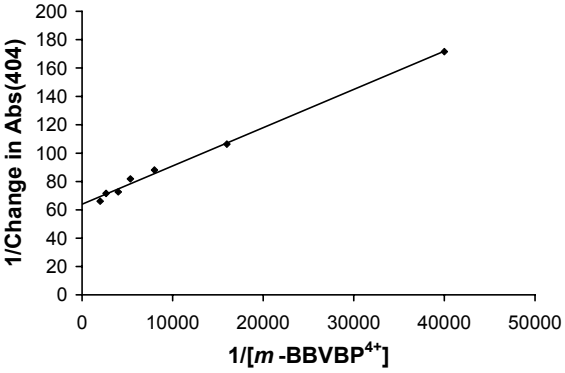




UV-Vis Absorbance Spectra and Benesi-Hildebrand plot of TSPP (4 x 10^{-6} M) in pH 7.4 buffer with increasing [*m*-$BBVBP^{4+}$].

MPTS:





UV-Vis Absorbance Spectra and Benesi-Hildebrand plot of MPTS (1 x 10^{-5} M) in pH 7.4 buffer with increasing [*m*-$BBVBP^{4+}$].

Fluorescence Emission Studies. Fluorescence intensity was taken as the area under the curve for all studies. Stern-Volmer constants were calculated by fitting the data with the equation:

$$(F/F_o) = (1 + K_s[Q])e^{V[Q]}$$

where V is the dynamic quenching constant, K_s is the static quenching constant, [Q] is the quencher concentration.

Table 1: Table of complete quenching data for all dyes studied.

DYE	**Static Quenching** K_{sv} (K_{uv})	**Dynamic Quenching** V
HPTS	50,000 ± 4,000 (45,000 ± 5,000)	3,800 ± 700
MPTS	22,000 ± 1000 (18,000 + 1,000)	300 + 90
PTCA	16,000 ± 4,000 (9,000 ± 1,000)	4,500 ± 200
APTS	19,400 ± 600 (14,000 ± 200)	1,900 ± 300
TCPP	14,400 ± 100 (22,000 ± 4,000)	0
TSPP	4,600 ± 100 (3,200 ± 400)	100 ± 40
$HPTS(Lys)_3$	10,900 ± 200 (15,000 + 4,000)	14,000 + 1,000
Fluorescein-SA	1,960 ± 70 (1,500 ± 600)	30 ± 10
Lucifer Yellow-I	260 ± 10*	
Sulforhodamine-B	110 ± 20*	
Sulforhodamine-101	120 ± 10*	
CTR	80 ± 10*	

K_{sv} (Static Stern-Volmer Quenching Constant determined from Fluorescence data); K_{uv} (Apparent Binding Constant for quencher-dye complexation from UV-vis data); V (dynamic or collisional quenching constant derived from fluorescence data); *Indicates a general quenching constant where the specific mode of quenching was not determined from graphical methods. Errors are reported as standard deviations based on three trials.

Apparent Glucose Binding Constants were calculated by fitting the data with the equation:

$$F_{calc} = (F_{min} + F_{max}K[\text{glucose}])/(1+ K[\text{glucose}])$$

where F_{calc} is the calculated fluorescence intensity; F_{min} is the initial fluorescence intensity of the quenched dye, F_{max} is the calculated intensity at which the fluorescence increase reaches its maximum. K is the apparent binding constant, and [glucose] is the concentration of glucose. [5]

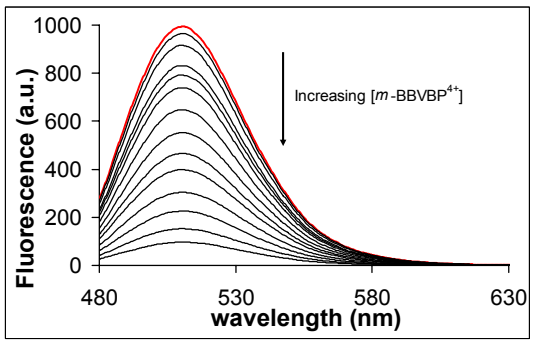
Quencher:Dye Ratio Optimization for Glucose Sensing. The fluorescence measurements were done *in situ* by taking the emission spectra of a 1:1 quencher:dye solution then adding an aliquot of buffered 1 M glucose solution and measuring the new fluorescence emission after shaking for 60 seconds. Additional aliquots were added and measurements taken until a glucose concentration of 30 mM was obtained. The overall process was then repeated at successively higher ratios until an optimal quencher:dye ratio could be determined.

Glucose Sensing. The fluorescence measurements for each dye were done *in situ* by taking the fluorescence emission spectra of the quencher:dye solution at its optimized ratio. An aliquot of buffered 1 M glucose solution was then added and the new fluorescence emission was measured after shaking for 60 seconds. Additional aliquots were added and measurements taken until a glucose concentration of 30 mM was obtained.

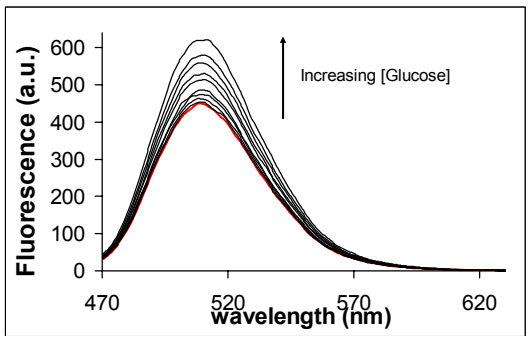
Representative Raw Fluorescence Emission Data for Quenching and Glucose Sensing Studies. For each dye, two fluorescence emission plots are shown: a) emission of the dye during titration with *m*-BBVBP^{4+} quencher; b) emission of the quenched dye during titration with glucose. The initial fluorescence emission for each titration is shown in red. Fluorescence intensity is given with arbitrary units (a.u.). Note: For each dye, two additional runs were done for both the quenching and glucose sensing titrations

HPTS:

a)

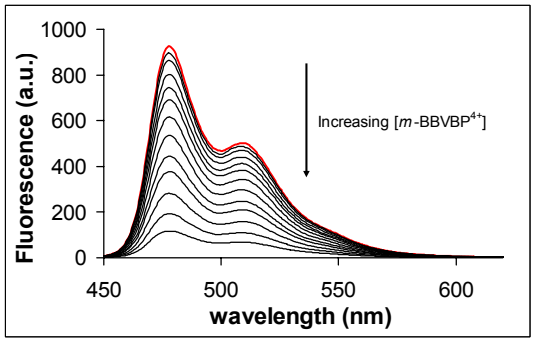


b)



PTCA:

a)



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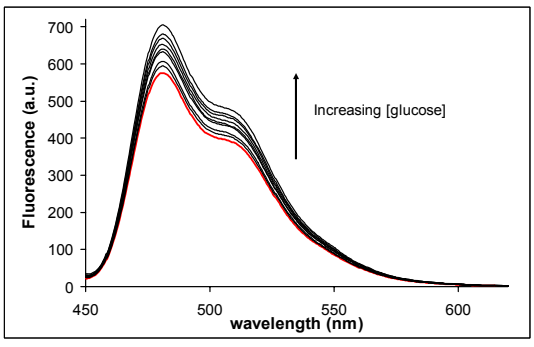
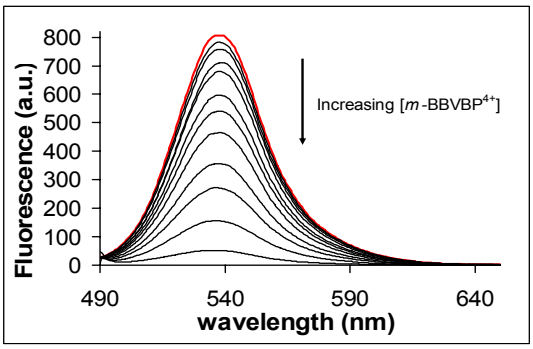


HPTS(Lys)$_3$:

a)



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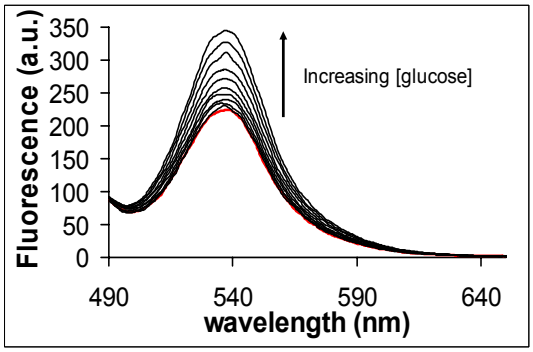
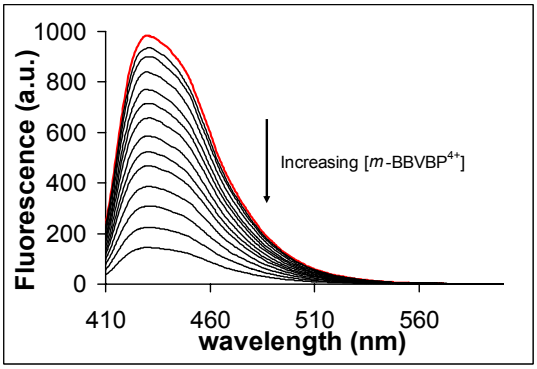


MPTS:

a)



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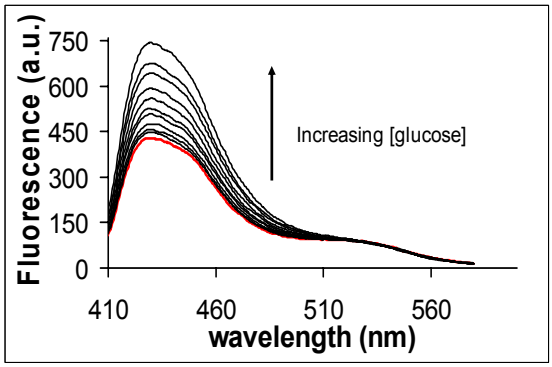
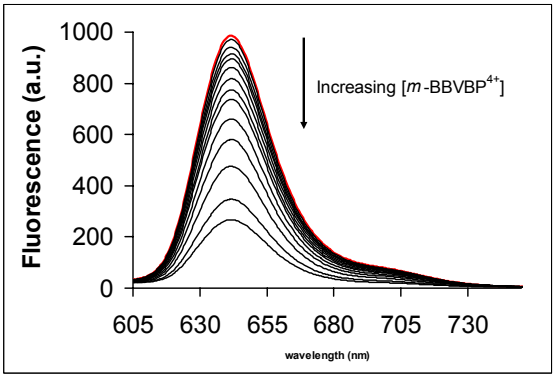


TSPP:

a)



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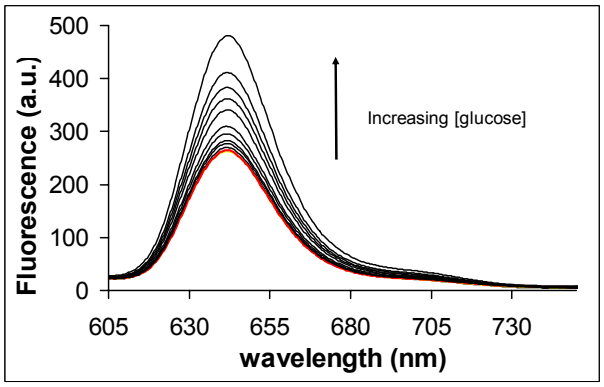
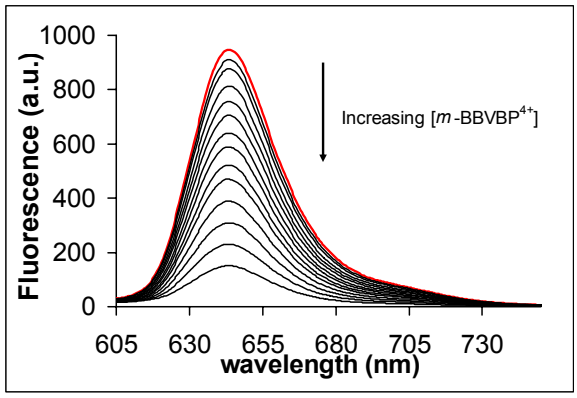


TCPP:

a)



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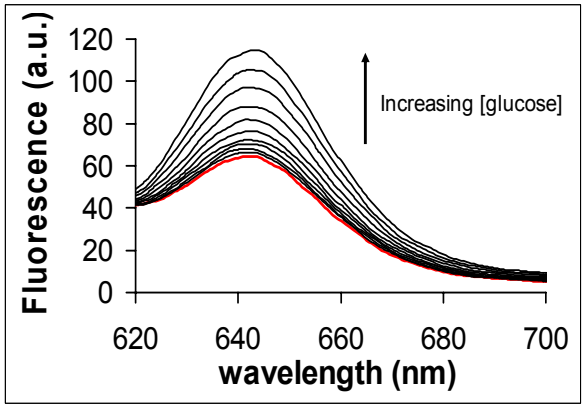
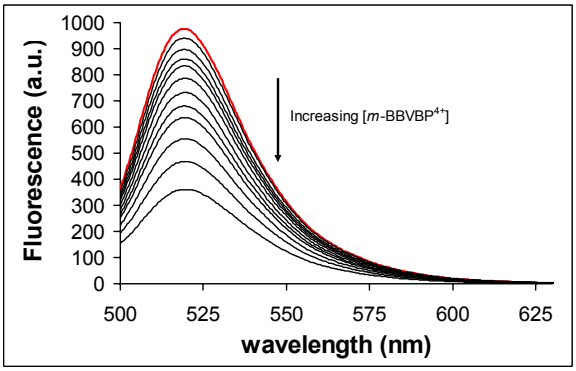


Fluorescein-SA:

a)



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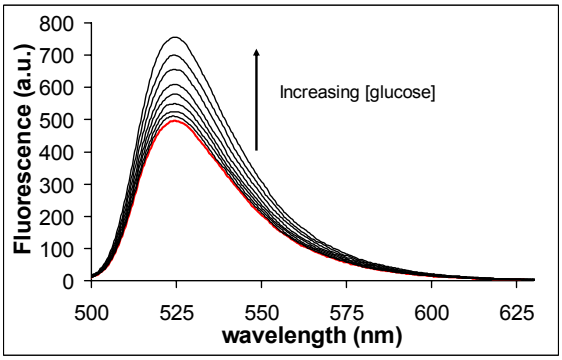
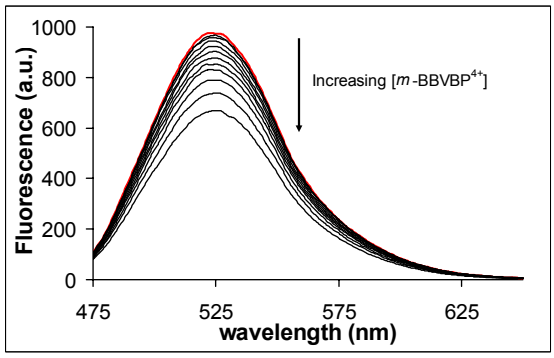


LY-I:

a)



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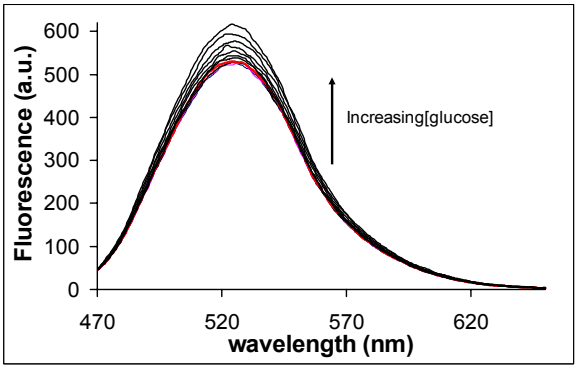
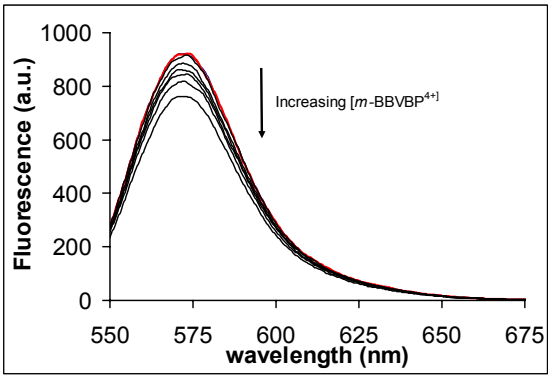


CTR:

a)



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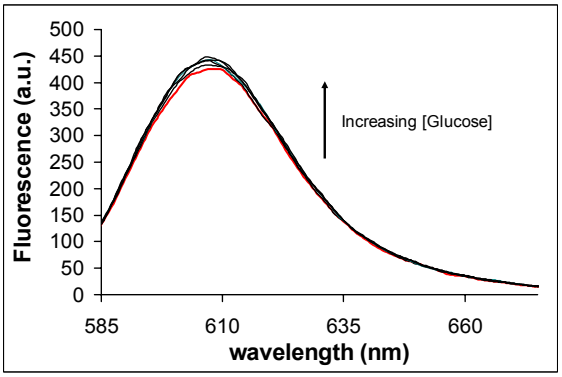
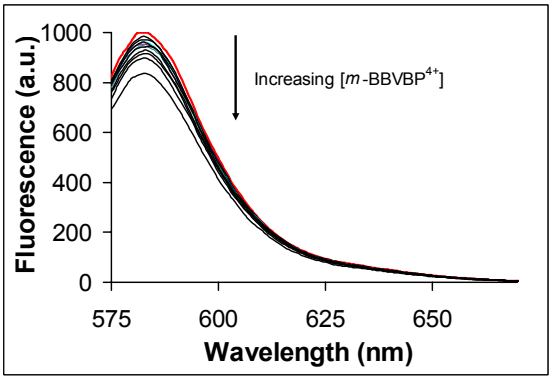
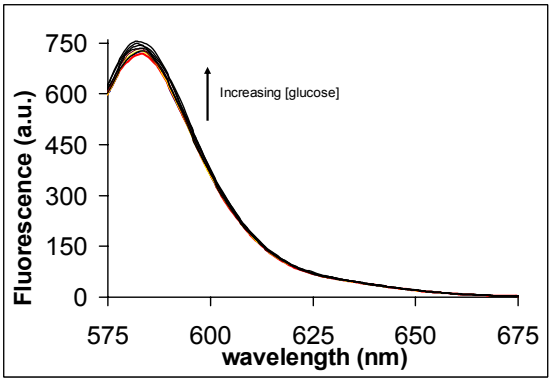


SR-B:

a)

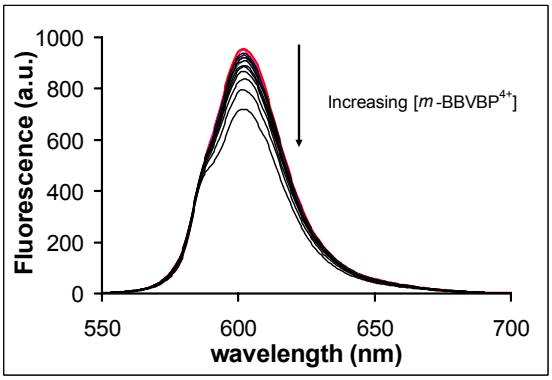


b)



SR-101:

a)



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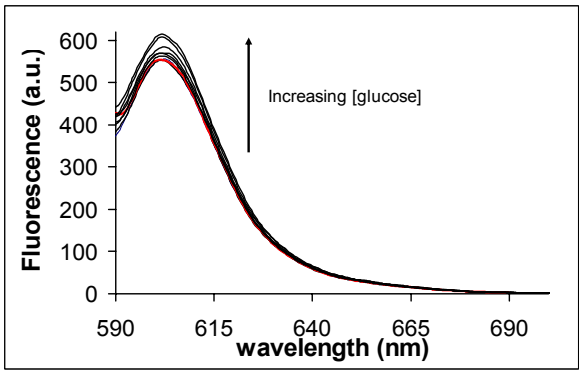

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