

## **New Optical Boron Detection Method**

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## Materials and Methods

2,3,6,7,10,11-hexahydroxytriphenylene hydrate (>95.0%) from TCI ([www.tcichemicals.com](http://www.tcichemicals.com)), boric acid ( $\geq 99.8\%$ ) from Merck ([www.merckmillipore.com](http://www.merckmillipore.com)), phenylboronic acid ( $\geq 97\%$ ) from Fluka ([www.sigmaaldrich.com](http://www.sigmaaldrich.com)), ammonium hydroxide 25% aqueous solution (puriss. p.a.) from Fluka, hydrochloric acid 37% aqueous solution (analytical, ACS, ISO reag.) from Riedel-de Haën ([www.sigmaaldrich.com](http://www.sigmaaldrich.com)), sodium hydroxide in pellets (purum) from Eka/AkzoNobel ([www.akzonobel.com](http://www.akzonobel.com)), and ethylenediaminetetraacetic acid (EDTA) disodium salt dihydrate (99-101%) from Riedel-de Haën were used as received. The aqueous solutions were prepared in demineralized water (Milli-Q).

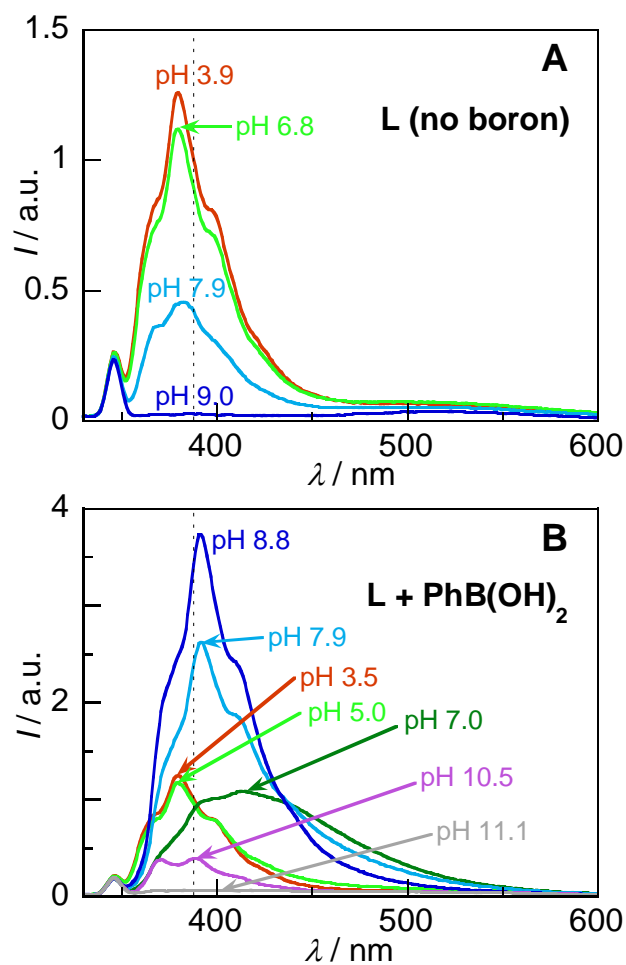
**Absorption and Fluorescence Measurements.** UV-visible absorption spectra were obtained in a JASCO V-660 spectrometer, using quartz cells ( $l = 1$  cm), unless indicated otherwise. The fluorescence spectra were recorded on a Horiba Jobin Yvon Fluorolog 3-22 spectrofluorimeter using quartz cells ( $l = 1$  cm), excitation slits with 3 nm bandwidth, emission slits with 5 nm bandwidth, integration time of 0.1 s/point, maximum sensitivity, and right angle mode. The emission spectra were recorded between 330 and 600 nm with excitation wavelength  $\lambda_{\text{exc}} = 310$  nm. The absorbance at the excitation wavelength was maintained lower than 0.1. All measurements were made at room temperature (23 °C).

**pH measurements.** pH was measured with a VWR pHenomenal pH1000L pH meter equipped with a VWR pHenomenal MIC 220 glass microelectrode and a VWR pHenomenal PT1000 1M temperature sensor.

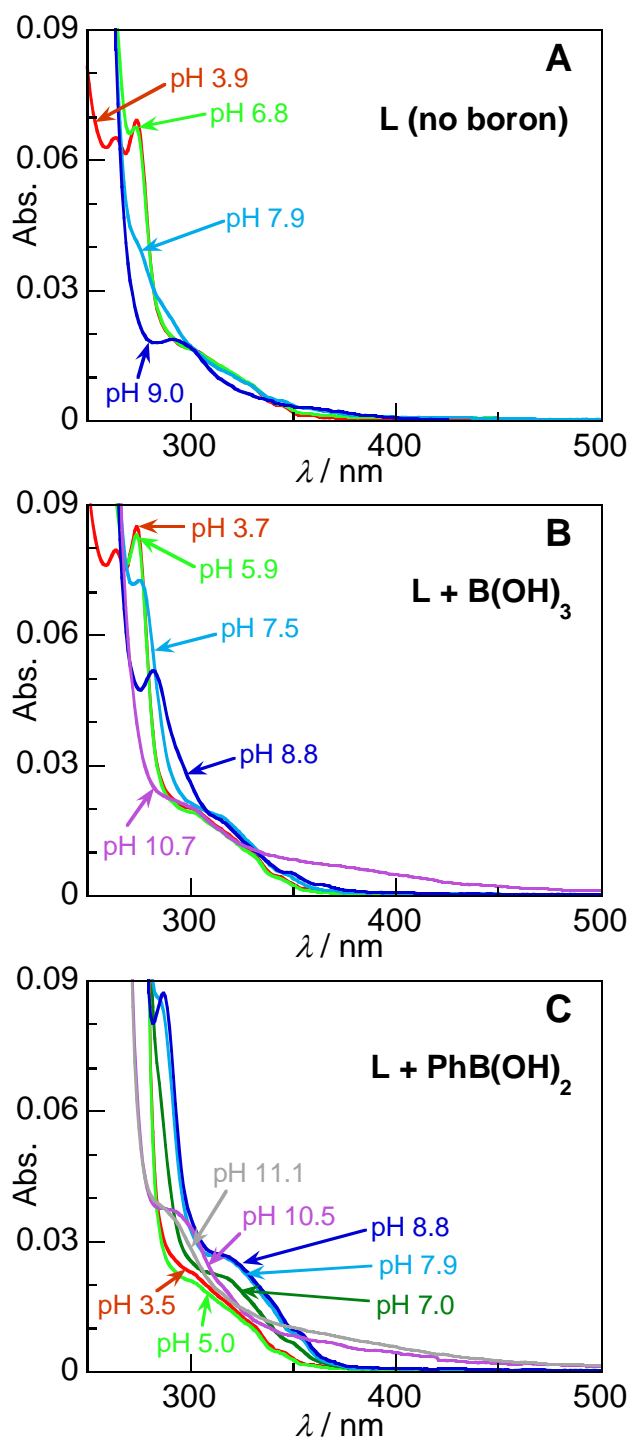
**Sensor performance.** 2,3,6,7,10,11-hexahydroxytriphenylene (**L**) was dissolved in water to a concentration of 1.25  $\mu\text{M}$  and stored in the refrigerator in a polypropylene flask. The samples were prepared in 2 mL polypropylene capped microtubes by adding 0.1 mL of boron solution or blank (water), 0.3 mL of a mixture of  $\text{NH}_3$  0.133 M and EDTA 0.067 M around the pre-defined pH (previously adjusted with HCl or NaOH), and 1.6 mL of 1.25  $\mu\text{M}$  **L** (final concentrations: **L** 1  $\mu\text{M}$ ,  $\text{NH}_3$  20 mM, EDTA 10 mM). The solutions were equilibrated in the dark for at least 2 h before the measurements. Care was taken to avoid contact between **L** solutions and borosilicate glass (including glass electrodes) until the final pH measurements.

**Assessment of boron concentration in the presence of both boric acid and phenylboronic acid.** Since the responses of the sensor to  $\text{B}(\text{OH})_3$  and  $\text{PhB}(\text{OH})_2$  are different, as the sensor has greater affinity for  $\text{PhB}(\text{OH})_2$ , in order to quantify boron in the presence of both boric acid and phenylboronic acid, we recommend the following procedure:

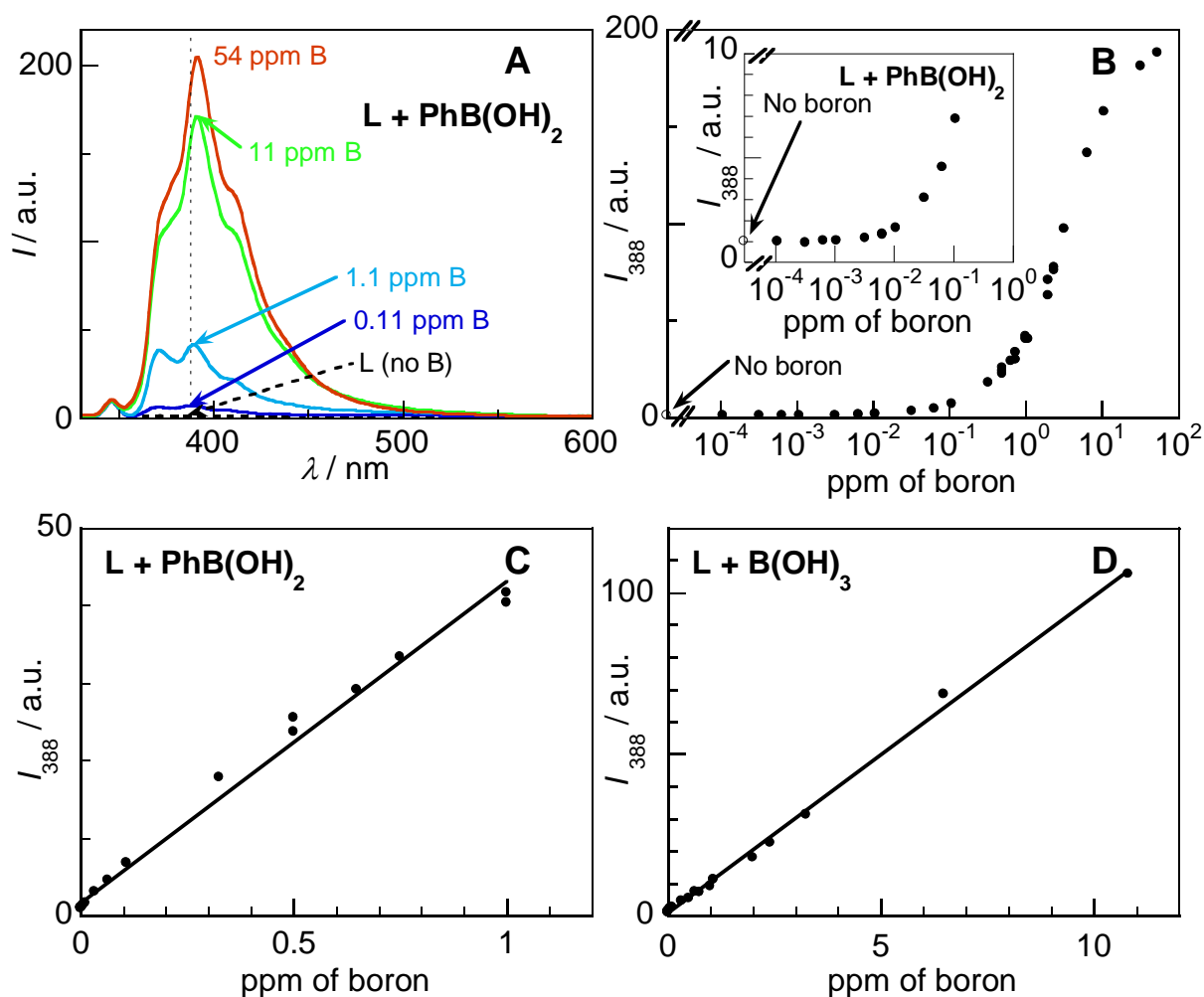
1. Quantify the concentration of phenylboronic acid in the sample from its absorption at 262 nm in the absence of sensor (absorption spectra: ESI, Fig. S7). We used ammonium buffer 0.02 M (in the absence of EDTA since its absorption overlaps that of phenylboronic acid). Boric acid does not absorb in the UV-vis region and therefore does not interfere with the results.
2. Prepare solutions with the concentration of phenylboronic acid determined in step 1 and increasing concentrations of boric acid. This calibration line can then be used to determine the amount of boric acid in the sample (ESI, Fig. S8 and S9).



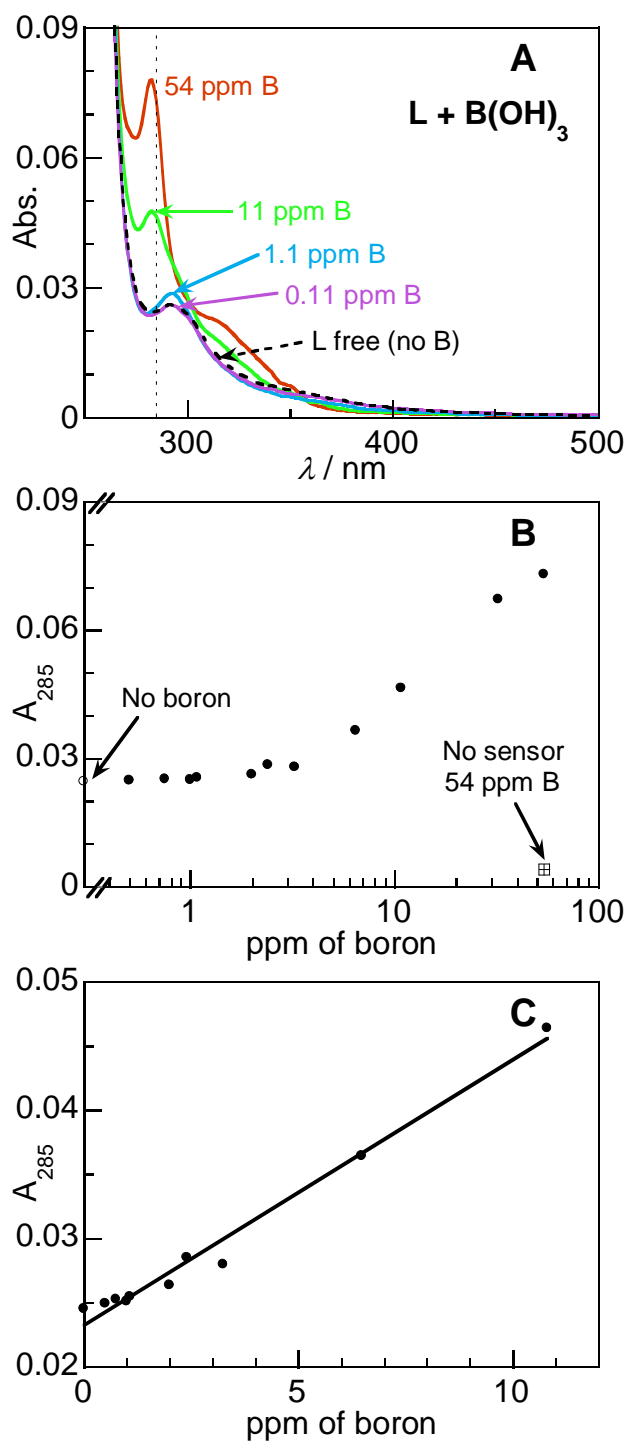
**Fig. S1.** Fluorescence emission spectra of **L** (thin dotted line:  $\lambda = 388$  nm) (A) in the absence of boron; and (B) in the presence of phenylboronic acid (1 mM, 11 ppm of boron). The spectra were recorded at  $\lambda_{\text{exc}} = 310$  nm in  $\text{NH}_3$  buffer (20 mM) in the presence of EDTA (10 mM) at  $T \approx 23$  °C. Fluorescence emission of free **L** is quenched at basic pH, but an enhancement of the fluorescence at ca.  $\text{pH} \approx 8 - 10$  is observed in the presence of phenylboronic acid (as well as of boric acid, as seen in Fig. 1).



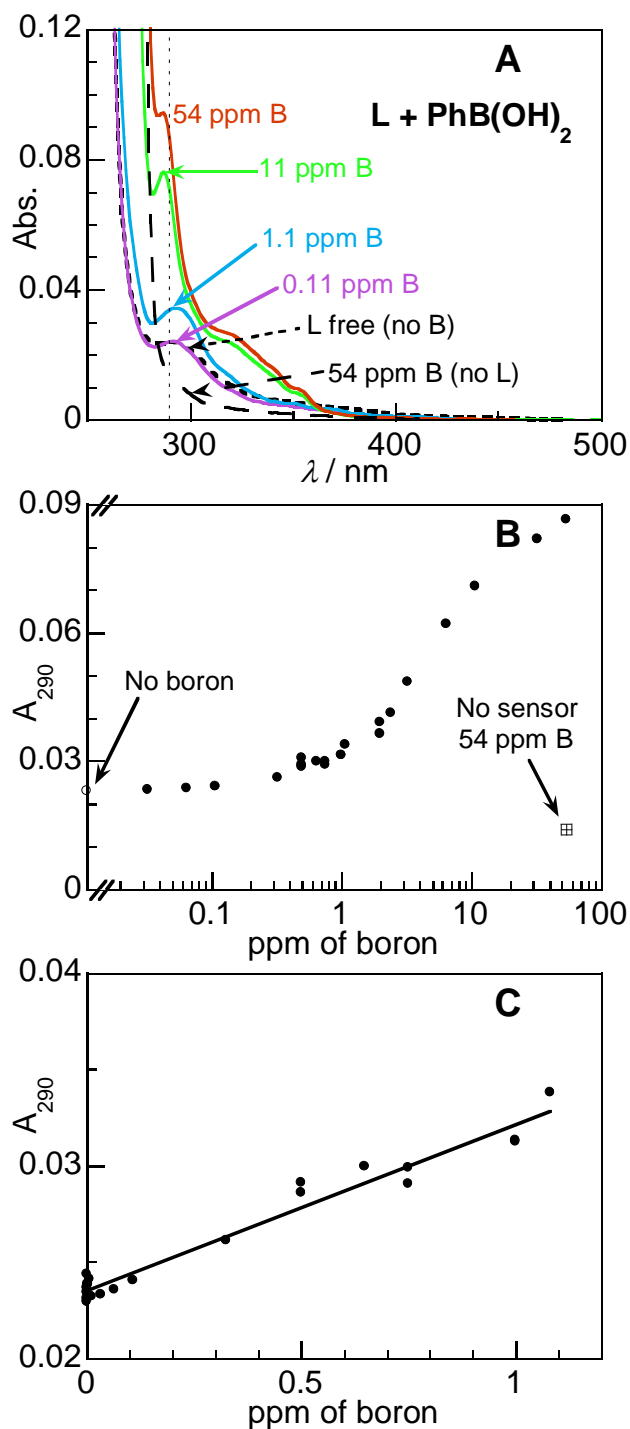
**Fig. S2.** UV-visible absorption spectra of **L** (A) in the absence of boron; (B) in the presence of boric acid (11 ppm of boron); and (C) in the presence of phenylboronic acid (11 ppm of boron). The spectra were recorded in  $\text{NH}_3$  buffer (20 mM) in the presence of EDTA (10 mM) at  $T \approx 23^\circ\text{C}$ .



**Fig. S3.** Fluorescence emission spectra (thin dotted line:  $\lambda = 388$  nm) (A) and the corresponding fluorescence titration curves at 388 nm (B) of **L** (1  $\mu\text{M}$ ) as a function of the concentration of phenylboronic acid (0 to 54 ppm of boron). (C) Example of calibration plot for phenylboronic acid ( $I_{388} = 41 \cdot [\text{B}] + 1.6$ ;  $r^2 = 0.991$ ; linearity was lost above 1 ppm of boron). (D) Example of calibration plot for boric acid ( $I_{388} = 9.8 \cdot [\text{B}] + 0.76$ ;  $r^2 = 0.995$ ; linearity was lost above 10 ppm of boron). Above the linearity zone, it is recommended that the analyte solution is diluted for more precise results, although concentration can be otherwise calculated by interpolation. The spectra were recorded at  $\lambda_{\text{exc}} = 310$  nm, 23  $^\circ\text{C}$ , in  $\text{NH}_3$  buffer (20 mM, pH = 9.1) in the presence of EDTA (10 mM).

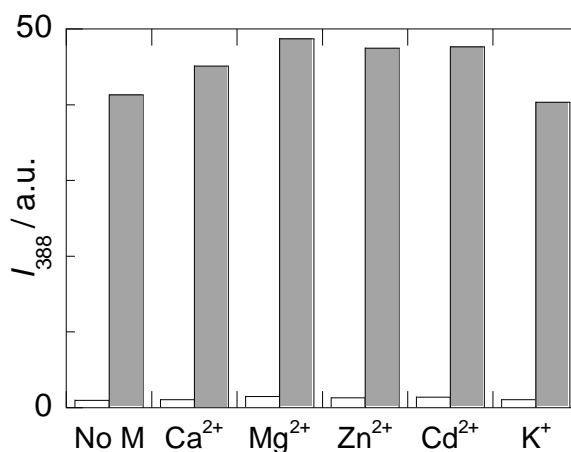


**Fig. S4.** UV-visible absorption spectra (thin dotted line:  $\lambda = 285 \text{ nm}$ ) (A), corresponding spectrophotometric titration curve at 285 nm (B) and example of calibration plot (C) ( $A_{285} = 0.0021 \cdot [B] + 0.0023$ ;  $r^2 = 0.98$ ; linearity was lost above 10 ppm of boron) of **L** ( $1 \mu\text{M}$ ,  $\text{pH} = 9.1$ ) as a function of the concentration of boric acid (from 0 to 54 ppm of boron). The spectra were recorded at  $23 \text{ }^\circ\text{C}$ , in  $\text{NH}_3$  buffer ( $20 \text{ mM}$ ,  $\text{pH} = 9.1$ ) in the presence of EDTA ( $10 \text{ mM}$ ).

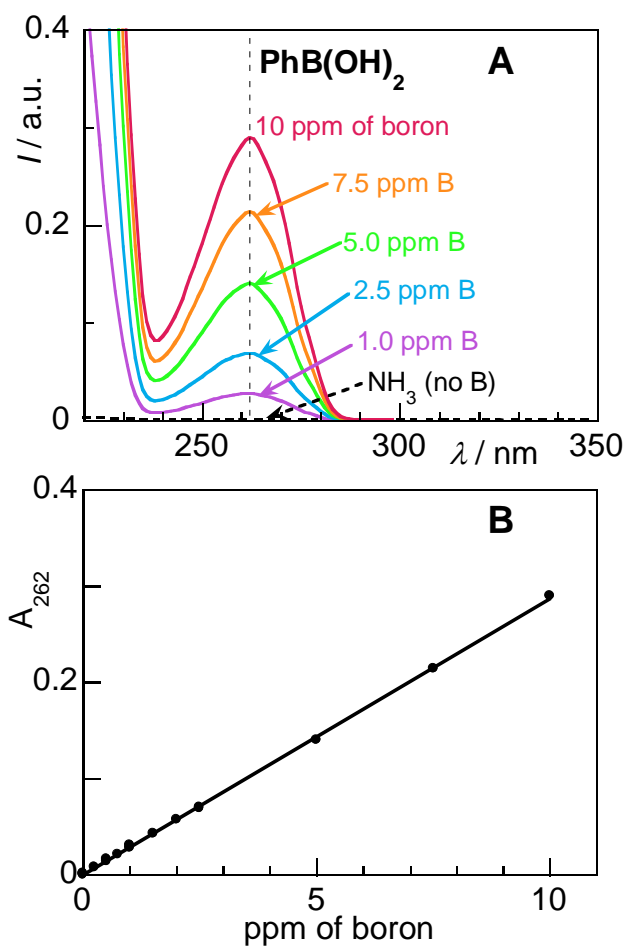


**Fig. S5.** UV-visible absorption spectra (thin dotted line:  $\lambda = 290 \text{ nm}$ ) (A), the respective spectrophotometric titration curve at 290 nm (B) and example of calibration plot (C) ( $A_{290} = 0.0086 \cdot [B] + 0.0024$ ;  $r^2 = 0.96$ ; linearity was lost above 1 ppm of boron) of **L** ( $1 \mu\text{M}$ ,  $\text{pH} = 9.1$ ) as a function of the concentration of phenylboronic acid (from 0 to 54 ppm of boron). The spectra were recorded at  $23 \text{ }^\circ\text{C}$ , in  $\text{NH}_3$  buffer ( $20 \text{ mM}$ ,  $\text{pH} = 9.1$ ) in the presence of EDTA ( $10 \text{ mM}$ ).

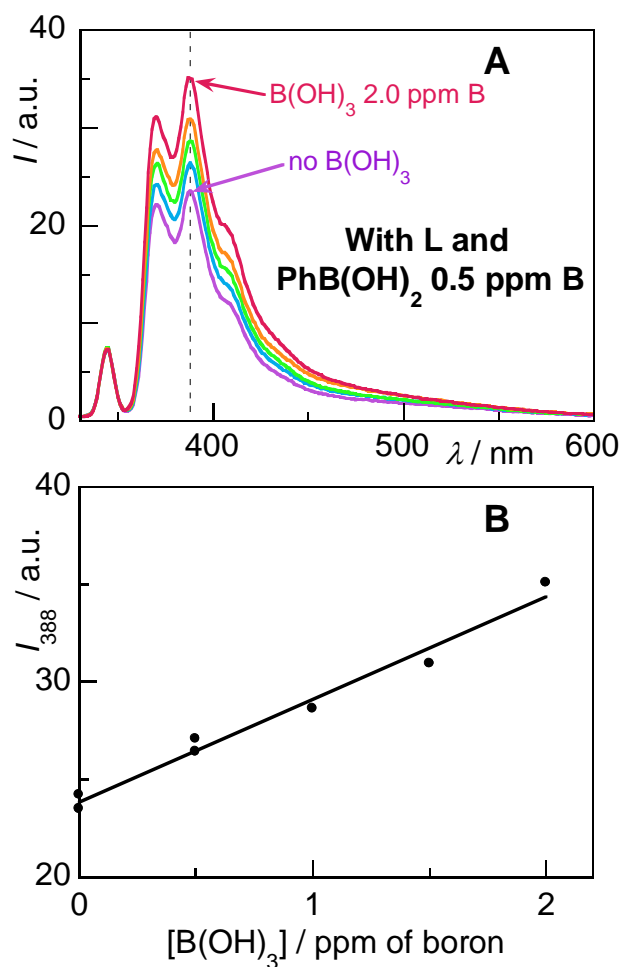




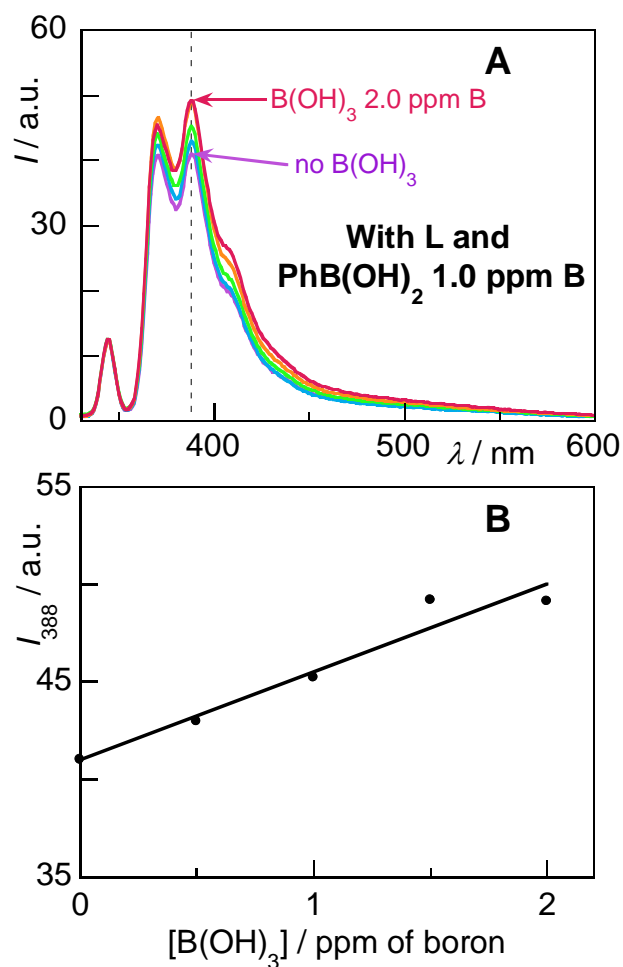
**Fig. S6.** Variation of the fluorescence intensity at 388 nm ( $\lambda_{\text{exc}} = 310$  nm) of **L** (1  $\mu\text{M}$ ) upon addition of phenylboronic acid in  $\text{NH}_3$  buffer (20 mM, pH = 9) in the presence of EDTA (10 mM) at  $T \approx 23$  °C: in the absence of interfering metal ions (*No M*) and after addition of 1000 equiv. of Ca(II), Mg(II), Zn(II), Cd(II), and K(I). In *white* for no added boron and in *grey* for added  $\text{PhB(OH)}_2$  (1 ppm of boron). The intensity of emission does not change significantly due to interference from the metal cations in the presence of EDTA. The small fluctuations are probably due to small differences in pH from deprotonation of EDTA upon complexation of the metal cations.



**Fig. S7.** UV-visible absorption spectra (thin dotted line:  $\lambda = 262$  nm) (A) and the respective spectrophotometric titration curve at 262 nm (B) (which can be used as calibration plot) of phenylboronic acid (from 0 to 10 ppm of boron;  $A_{262} = 0.0029 \cdot [B] + 0.0005$ ;  $r^2 = 0.9995$ ). The spectra were recorded at 23 °C, in NH<sub>3</sub> buffer (20 mM, pH = 9.1).



**Fig. S8.** Fluorescence emission spectra (thin dotted line:  $\lambda = 388$  nm) (A) and the corresponding fluorescence titration curve at 388 nm (B) of **L** ( $1 \mu\text{M}$ ) as a function of the concentration of boric acid (0 to 2 ppm of boron) in the presence of phenylboronic acid at the concentration of 0.5 ppm of boron ( $I_{388} = 5.3 \cdot [\text{B(OH)}_3] + 24$ ;  $r^2 = 0.99$ ). This can be used to assess the amount of boric acid, for a pre-determined concentration of phenylboronic acid of 0.5 ppm of boron. The spectra were recorded at  $\lambda_{\text{exc}} = 310$  nm,  $23$  °C, in  $\text{NH}_3$  buffer (20 mM,  $\text{pH} = 9.1$ ) in the presence of EDTA (10 mM).



**Fig. S9.** Fluorescence emission spectra (thin dotted line:  $\lambda = 388$  nm) (A) and the corresponding fluorescence titration curve at 388 nm (B) of **L** ( $1 \mu\text{M}$ ) as a function of the concentration of boric acid (0 to 2 ppm of boron) in the presence of phenylboronic acid at the concentration of 1.0 ppm of boron ( $I_{388} = 4.5 \cdot [B(OH)_3] + 41$ ;  $r^2 = 0.97$ ). This can be used to assess the amount of boric acid, for a pre-determined concentration of phenylboronic acid of 1.0 ppm of boron. The spectra were recorded at  $\lambda_{\text{exc}} = 310$  nm,  $23$  °C, in  $\text{NH}_3$  buffer (20 mM,  $\text{pH} = 9.1$ ) in the presence of EDTA (10 mM).