Detection of Boronic Acids through Excited-State Intramolecular Proton-Transfer Fluorescence

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Materials

Silica-coated thin layer chromatography plates were obtained from Macherey Nagel (Duren, Germany). All reagent-grade materials were from Sigma–Aldrich (St. Louis, MO) and were used without further purification, except for phenylboronic acid, benzoxaborole, and 3-nitrophenylboronic acid, which were from Combi-Blocks (San Diego, CA).

General Methods

Image Capture. The images in Figures 2 and 3 were acquired with a Nikon digital SLR camera under identical exposure times. Technical details are provided in the Camera Settings section. Bright-field and epifluorescent microscopy images of agarose beads were performed on an upright microscope equipped with a CCD camera with 4× magnification. Epifluorescence images were taken by illuminating at ~365 nm using a handheld UV lamp.

Experimental Methods

Spotting for Figure 2. Alizarin Red Stain was prepared and applied according to the previous report of Duval and coworkers.[1] A 1.0 mM solution was prepared by adding 0.10 mmol of Alizarin to 0.10 L of anhydrous acetone. Optimal visualization was accomplished by slow immersion of silica-coated glass TLC plates in the alizarin solution, removal, and then allowing the stain to dry completely at room temperature. Plates were then viewed by illuminating the beads at ~365 nm using a handheld UV lamp.

A 1.0 mM solution of HBQ stain was prepared by adding 0.10 mmol of 10-hydroxybenzo[*h*]quinoline to 0.10 L of absolute ethanol. Optimal visualization was accomplished by immersion of silica-coated glass TLC plates in the HBQ solution, and then

allowing the stain to dry completely by applying a standard laboratory heat gun. Plates were then viewed by illuminating at ~365 nm using a handheld UV lamp.

A 10 mM solution of phenylboronic acid was prepared by addition to methanol. Subsequent dilutions were prepared in methanol from the original 10 mM stock.

Spotting for Figures 3 and 4. All positive controls were prepared as solutions at 10 mM concentrations in methanol. All compounds tested were applied as 2-μL samples to a glass backed TLC plate and allowed to air-dry at room temperature. Negative control samples were prepared in methanol at concentrations required for sufficient visualization following absorbance at 254 nm, prior to dye treatment, and allowed to air-dry at room temperature.

A 1.0 mM solution of Alizarin in acetone was prepared. Visualization was accomplished by slow immersion of silica-coated glass TLC plates in the alizarin solution, removal, and then allowing the stain to dry completely at room temperature. Plates were then viewed by illuminating at ~365 nm using a handheld UV lamp

HBQ stain was prepared by adding 0.10 mmol of 10-hydroxybenzo[*h*]quinoline to 0.10 L of absolute ethanol. Detection was performed by briefly immersing the TLC plate in solution, then drying the plate using a standard laboratory heat gun. Plates were viewed by illuminating at ~365 nm using a handheld UV lamp.

Detection of Boronic Acids on Agarose Support. The following preparation was performed on Agarose beads (6% cross-linking) with and without bound *m*-aminophenylboronic acid. Agarose beads (50 mg) were suspended in methanol and filtered. The beads were resuspended in 1.0 mL of HBQ stain solution for 1 min and filtered and washed with 5 mL EtOH. The beads were dried and imaged using microscopy.

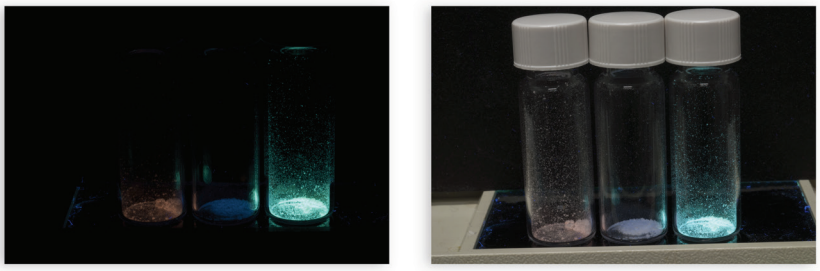

Figure S1. Agarose beads viewed "in vial" by illuminating at ~365 nm using a handheld UV lamp without (left image) supplemental lights, and with (right image) overhead lighting. Non-functionalized agarose treated with HBQ (left, "orange"), 5-amino phenylboronic acid crosslinked agarose, untreated (center), and 5-amino phenylboronic acid functionalized agarose following treatment with HBQ (right, "blue-green fluorescent").

HBQ fluorescence relationship with phenylboronic acid (Figure 6). Fluorescence intensity was measured with an Infinite M1000 Pro microplate reader (Tecan Group, Mannedorf, Switzerland). Phenylboronic acid (PBA) was prepared from a 20 mM stock in methanol, as described previously. PBA solution (20 μL) was applied to cover the bottom of each well of a sterile 96-well plate (Costar black plate, clear bottom with lid from Corning, Tewksbury, MA). To ensure that HBQ stain was in excess, to each sample was added 50 μL of a 10 mM HBQ solution in ethanol for 20, 10, and 5 mM PBA, and 20 μL of a 1 mM HBQ solution in ethanol for other dilutions of PBA. The resulting solutions were allowed to dry completely before analysis.

Bottom-up fluorescence was measured in triplicate for each dilution. Excitation was applied at 365 nm with a 5.0-nm bandwidth. Emission was detected at 500 nm with a 5.0-nm bandwidth to minimize oversaturation of the detector. Multiple reads per well were measured in a 4 × 4 filled circle with a 100-µm border, and then averaged for each sample well. Average values of fluorescence intensity were plotted with the program Prism from GraphPad (La Jolla, CA). Error bars were determined based on standard deviation from average values.

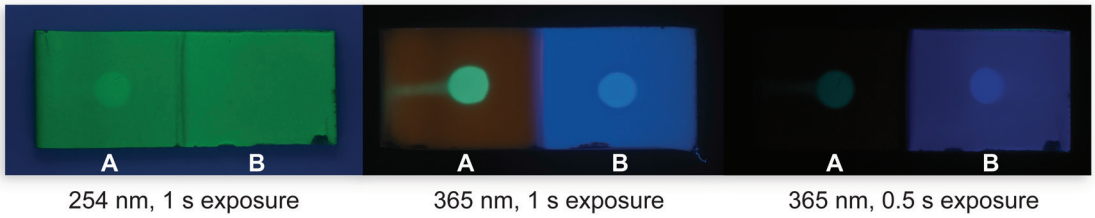

Figure S2. Comparison of 10-hydroxybenzo[*h*]quinolone (HBQ) to 2-(2′-hydroxyphenyl)benzimidazole.[2] phenylboronic acid in methanol (2 µL of a 10 mM solution) was spotted onto two halves of a glass-backed TLC silica plate and air-dried. The plate was dipped on one side into a 1 mM solution of HBQ in ethanol (A), or a 1 mM solution of 2-(2′-hydroxyphenyl)benzimidazole in ethanol (B). The plate was then dried using a heating gun, and visualized by illuminating at a short (~254 nm) or long (~365 nm) wavelength using a handheld UV lamp. 2-(2′-Hydroxyphenyl)benzimidazole was judged to be inferior as a stain due to the mildly discernable small stokes shift between the ESIPT-on and ESIPT-off emissions compared to those of HBQ.

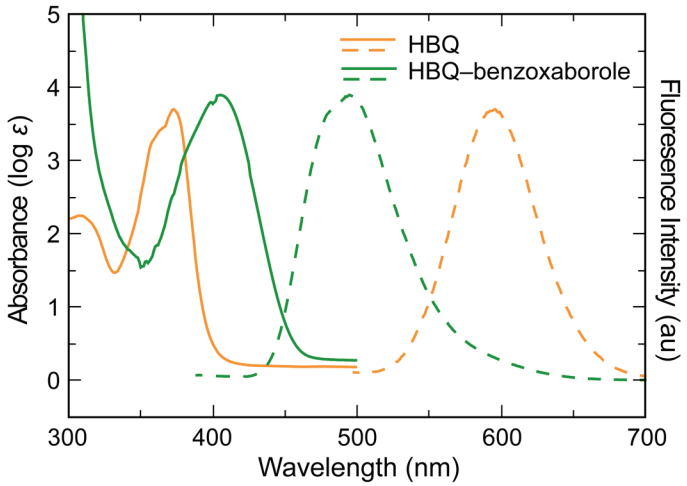

Figure S3. Absorption and emission spectra of HBQ and its complex with benzoxaborole. Fluorescence spectra were recorded in $CHCl_3$ with a Photon Technology International 810 fluorometer using right-angle detection. Ultraviolet–visible absorption spectra were measured with a Varian Cary 300 Bio diode array spectrophotometer and corrected for background signal with a solvent-filled cuvette. The instability of the HBQ–benzoxaborole precluded determination of its quantum yield.[3]

Camera Settings

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References

(1) Duval, F.; van Beek, T. A.; Zuilhof, H., *Synlett* **2012**, 1751–1754.

(2) Benelhadj, K.; Massue, J.; Retailleau, P.; Ulrich, G.; Ziessel, R., *Org. Lett.* **2013,** *15*, 2918–2921.

(3) A crystal structure of the HBQ–benzoxaborole complex is reported in Robin, B.; Buell, G.; Kiprof, P.; and Nemykin, V. N. *Acta Crystallogr. Sect. E.*, **2008**, *64*, o314–o315. Additional relevant spectral data are reported in Chou, P.; Chen, Y.; Yu, W.; Chou, Y.; Wei, C.; Cheng, Y. *J. Phys. Chem. A* **2001**, *105*, 1731–1740.