Identification of Carboxylic and Organoboronic Acids and Phenols with a Single Benzobisoxazole Fluorophore

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Supporting Information

General Methods

Carboxylic acids **C1**–**C12** (and **C13**–**C16**, vide infra), organoboronic acids **B1**–**B9**, and phenols **P1**–**P12** (and **P13**–**P14**, vide infra) were purchased from commercial suppliers and used without further purification. Solvents were reagent grade and were used as received. Cruciform **1** was prepared according to literature procedure.[S1]

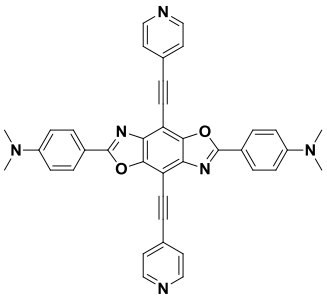

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Figure S1. Cruciform **1**.

Photographs of emission colors were taken using a FujiFilm FinePix S9000 digital camera, with a shutter speed of 0.5 s. A handheld UVLS-28 EL series UV lamp ($\lambda_{excitation}$ = 365 nm) was used as the light source. All photographs were taken in a dark windowless room, with a 45 cm (18 in) distance between the sample cuvettes and the camera lens. A photograph and the schematics of the photography setup are shown in Figure S2.

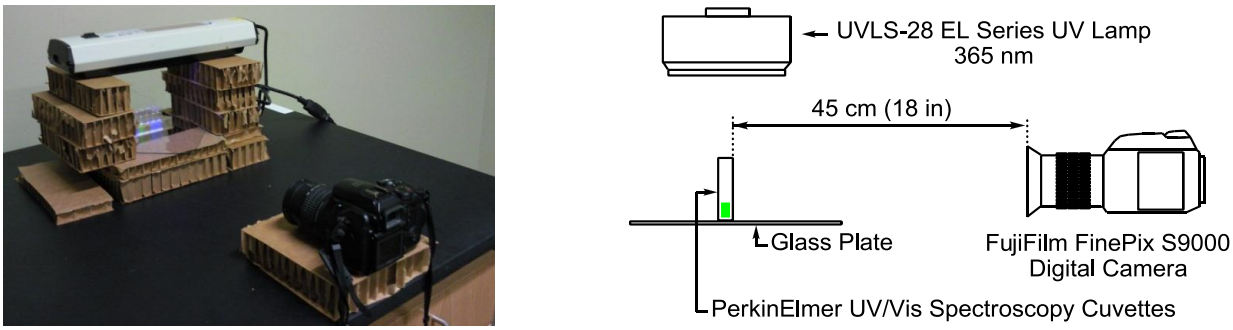

Figure S2. Darkroom setup for taking emission color photographs.

Experiments are presented in the order that follows the discussion of the manuscript. Compound numbers are identical to those in the main text of the manuscript.

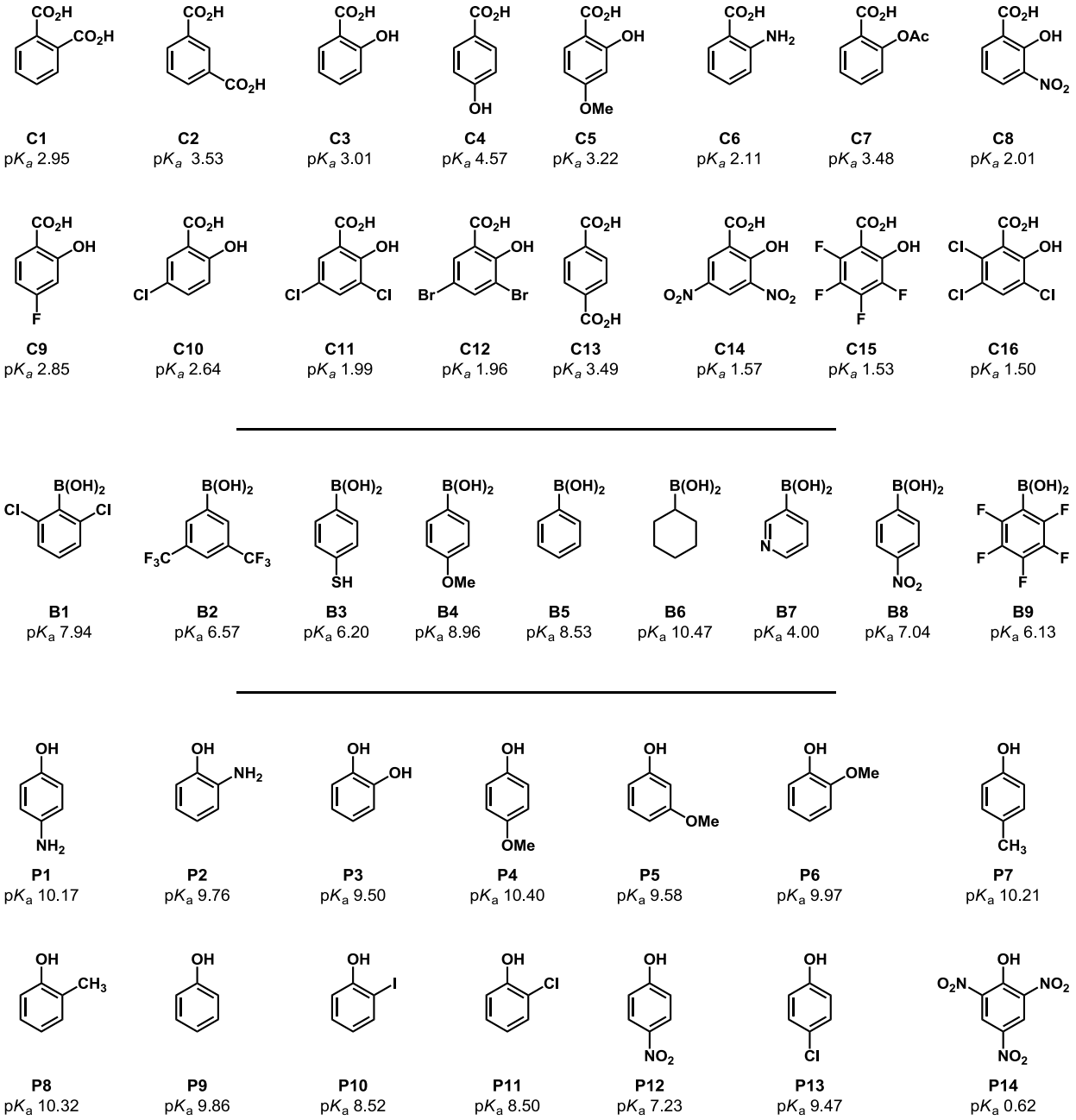

Figure S3. Sixteen carboxylic acids **C1**–**C16**, nine boronic acids **B1**–**B9**, and fourteen phenols **P1**–**P14** were analyzed in this work. All carboxylic and boronic acids, and phenols **P11**–**P14** were analyzed using cruciform **1** as the sole sensor. Phenols **P1**–**P12** were identified using solutions of cruciform **1** in combination with **B1** and **B5** as additives.

Discrimination of C1–C16, B1–B9, and P11–P14 Using Solutions of Cruciform 1

For each analyte, five individual solutions were prepared by dissolving 50 mg of the analyte in 3 mL each of acetonitrile (AN), 1,2,4-trichlorobenzene (TCB), dichloromethane (DCM), cyclohexane (CH), and chlorobenzene (CB). Thus, the resultant concentration of analytes in

these solutions was ~16.7 g L^{-1}. Next, 1.8 mL of these solutions was transferred into a 10×10 mm quartz cuvette, followed by a 20 µL injection of the 1.0×10^{-4} M solution of cruciform **1** in DCM (2.0×10^{-9} mol). The mixed solution was stirred using a disposable pipette. This procedure was repeated for each of the five solvents, and a five-cuvette set was then placed on glass plate and irradiated by a handheld UV lamp (365 nm). A digital photograph was immediately taken.

Discrimination of P1–P12 with Solutions of Cruciform 1 Combined with B1 or B5

Each of the phenol analytes **P1**–**P12** was treated with ten separate sensing solutions.

First five solutions were prepared by dissolving phenylboronic acid (**B5**, 48.8 mg, 0.40 mmol) in 20 mL of 1.0×10^{-6} M solutions of cruciform **1** in AN, TCB, CH, DCM, and chloroform (CF), successively. Second five solutions were prepared by dissolving 2,6-dichlorophenylboronic acid (**B1**, 76.3 mg, 0.40 mmol) in 20 mL of 1.0×10^{-6} M solutions of cruciform **1** in AN, TCB, CH, DCM, and CF, successively. In each of these ten sensor solutions, the molar ratio between the boronic acid and cruciform **1** was 20,000 : 1.

Sensing solutions were left to stand for 7 h, before dissolving ten samples of 20 mg each of phenol derivatives **P1**–**P12** (Figure S3) in 1 mL of each of the sensing solutions. The final molar ratios of phenol : boronic acid : **1** varied between ~90,500 : 20,000 : 1 to ~213,000 : 20,000 : 1.

All photos were taken 4 h after the corresponding phenol/(**B1** or **B5**)/**1** solutions were prepared. Two five-cuvette sets (one for **B1**, one for **B5** additive) were placed on glass plate, irradiated at 365 nm by a handheld UV lamp, and immediately photographed.

Calculation of RGB Changes

From each of the digital photographs of emission colors, representative 180×180 pixel segments were cut out using Adobe PhotoShop. These cut-outs were arranged into panels (as shown in Figure S4) using CorelDRAW X3, and then exported into TIFF files. Figure S5 shows

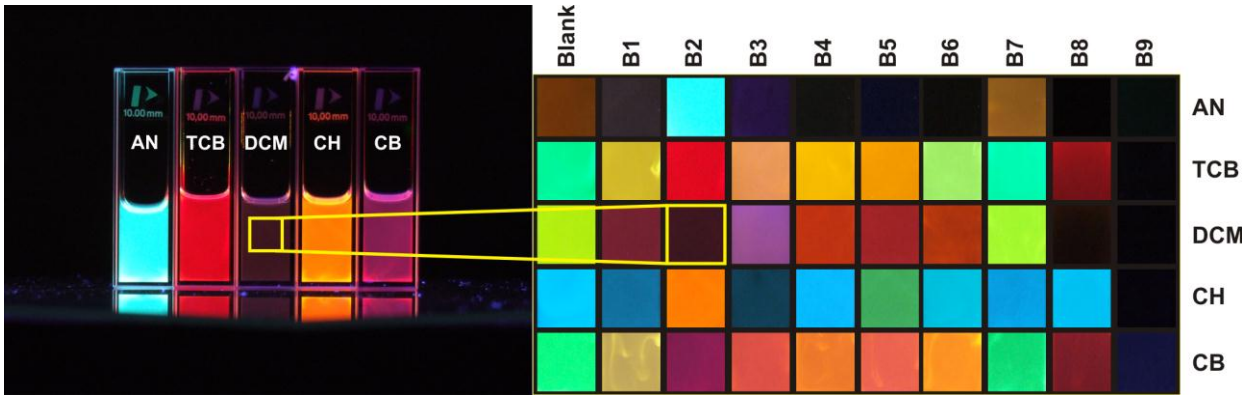

Figure S4. The original photo of emission colors of five **B2** solutions in UV/Vis spectroscopy cuvettes (left) and the panel of all boronic acids' emission colors in five solvents (right).

the complete panels of emission colors of pure cruciform **1** exposed to carboxylic (**C1**–**C16**) and boronic (**B1**–**B9**) acids, and phenols **P11**–**P14**. Figure S6 shows the corresponding panel for sensing of phenols **P1**–**P12** using the combination of cruciform **1** and boronic acids **B1** and **B5**.

Numeric alues for R(ed), G(reen), and B(lue) colors were extracted from these panels using freely downloadable Colour Contrast Analyzer.[S2] These values were extracted for each analyte in each solvent, and were then statistically treated and compared to other analytes and blank solutions using Microsoft Excel. The Excel file containing the original analysis is a separate part of the Supporting Information.

Specifically, for each analyte we calculated standard deviations σ of its R/G/B values from those assigned to the blank solution of **1**, or **1** + **B1**/**B5** in the same solvent. On the example of analyte **C1** in acetonitrile (AN), this standard deviation $\sigma_{\mathbf{C1}@\mathrm{AN}}$ was calculated as:

$$\sigma_{\boldsymbol{C1}@AN} = \sqrt{\frac{(R_{\boldsymbol{C1}}^{AN} - R_{\boldsymbol{1}}^{AN})^2 + (G_{\boldsymbol{C1}}^{AN} - G_{\boldsymbol{1}}^{AN})^2 + (B_{\boldsymbol{C1}}^{AN} - B_{\boldsymbol{1}}^{AN})^2}{3}},$$

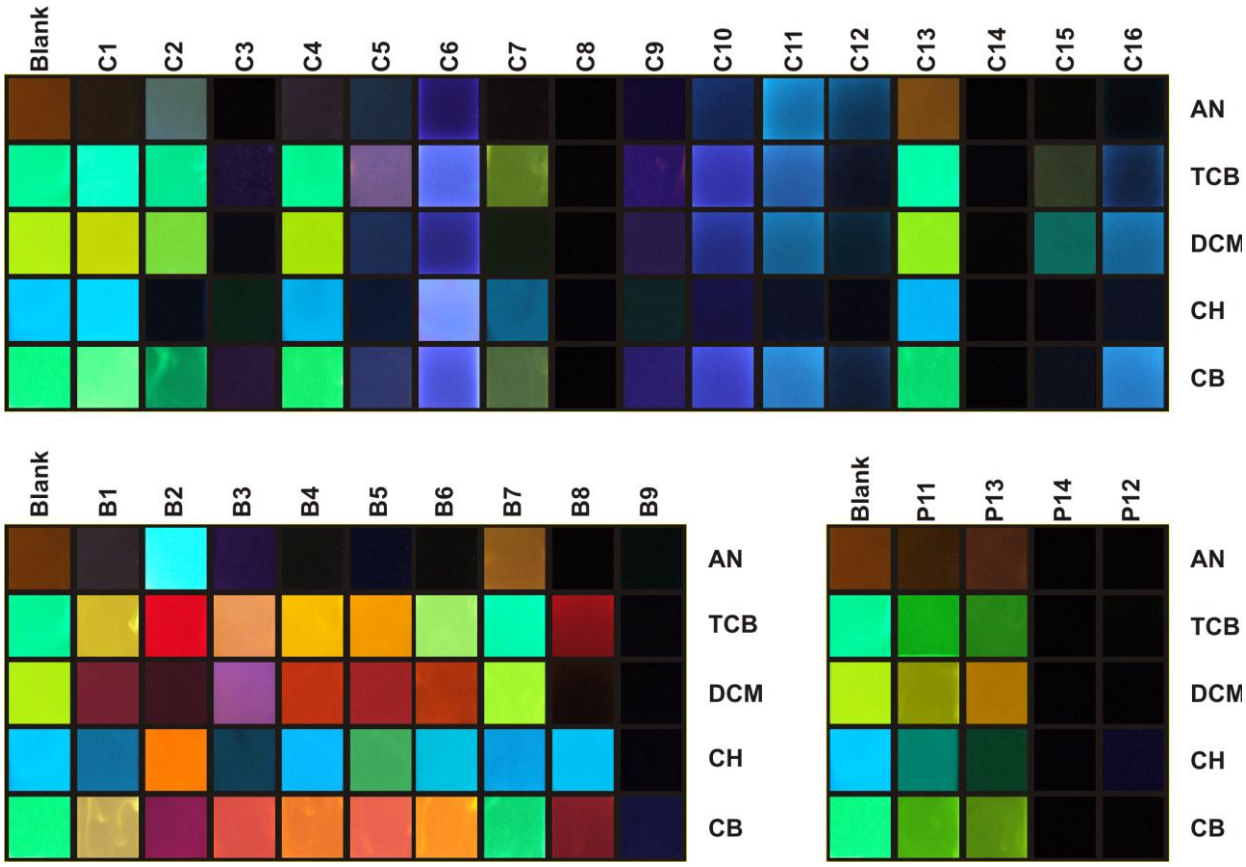

Figure S5. Panels with the photographs of the emission colors observed when the solutions of cruciform **1** were exposed to carboxylic acids **C1**–**C16** (top), boronic acids **B1**–**B9** (bottom left), and phenols **P11**–**P14** (bottom right), and irradiated at 365 nm.

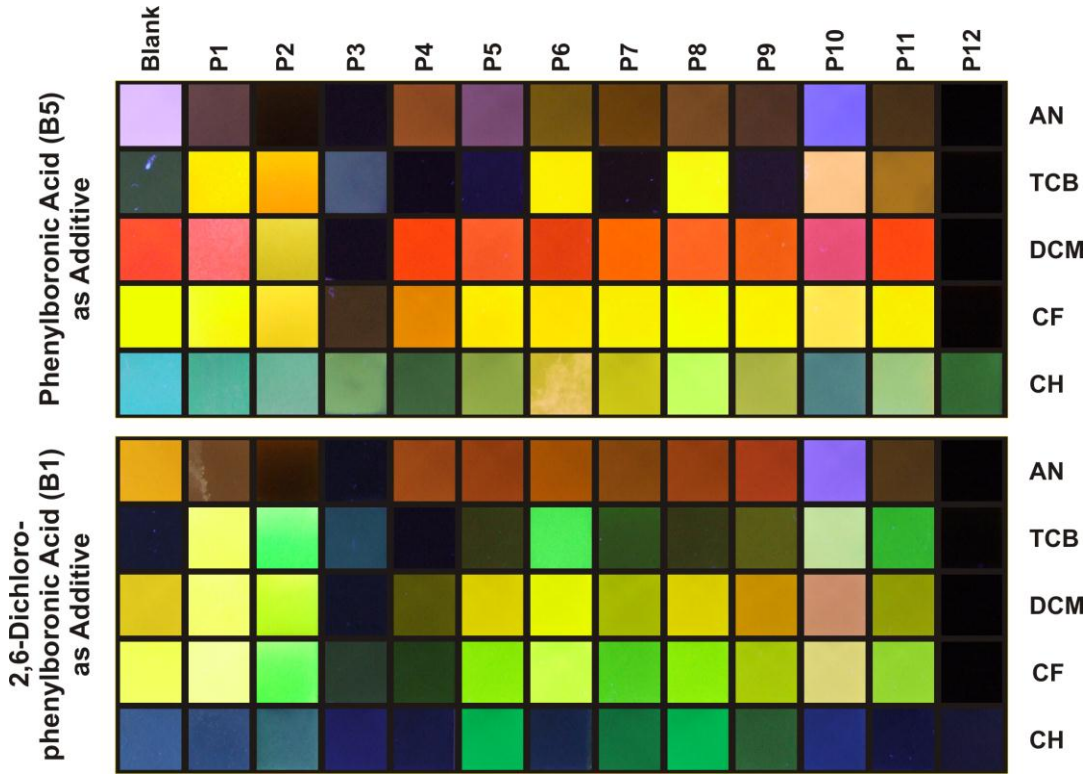

Figure S6. Panels with the photographs of the emission colors observed when the solutions of cruciform **1** and **B5** (top) and **B1** (bottom) were exposed to phenols **P1**–**P12**, and irradiated at 365 nm.

where R_{C1}^{AN} represented the R value of **C1** in AN, and R_{1}^{AN} represented the R value of cruciform **1** in AN (and analogously for G and B values). All of the obtained σ values are given in the attached Excel file.

To evaluate the correlation between the R/G/B values of different analytes with the same compound class, we also calculated relative standard deviations σ'. On an example of compounds **C1** and **C2**, this $\sigma'_{C1@C2}$ was defined as:

$$\sigma'_{C1@C2} = \sqrt{\frac{\sum_{solv}^{i}(R_{C1}-R_{C2})^2+(G_{C1}-G_{C2})^2+(B_{C1}-B_{C2})^2}{3*i}}.$$

Importantly, relative standard deviations are not solvent-specific, as they statistically treat R/G/B differences across all examined solvents. Thus, a single number—plotted in the graphs in Figures 2 (for **C1**–**C12**), 3 (for **B1**–**B9**), and 4 (for **P1**–**P12**, using boronic acid additives) in the manuscript—describes a difference between the two analytes.

Figure S7 shows the correlation diagram for discrimination of phenols **P11**–**P14** from each other using only the solution of cruciform **1** (i.e. without boronic acids). Correlation values are significantly lower than in the cases of carboxylic and boronic acids. In addition, cruciform **1** alone poorly distinguishes between **P11**/**P13** and **P12**/**P14** pairs.

Note on the Use of ImageJ Image Subtraction Routines

Even though we did not use image manipulation program ImageJ[S3] in our analysis, this program is a common and useful tool in image manipulations required for this and similar studies. For example, instead of individually subtracting R/G/B values of the emission colors of the blank solutions of **1** from those of the corresponding emission colors of solutions of **1** with added analytes, ImageJ could simply subtract the two images and produce a composite with differential R/G/B values. We feel it is necessary to include a cautionary note on the potentially different results that similar ImageJ commands can produce. To illustrate this issue, we performed several exemplary manipulations of our data, using our panel of emission colors (Figure S8, left), and the corresponding "mask image", which consisted only of blank cruciform samples' emission colors (Figure S8, right).

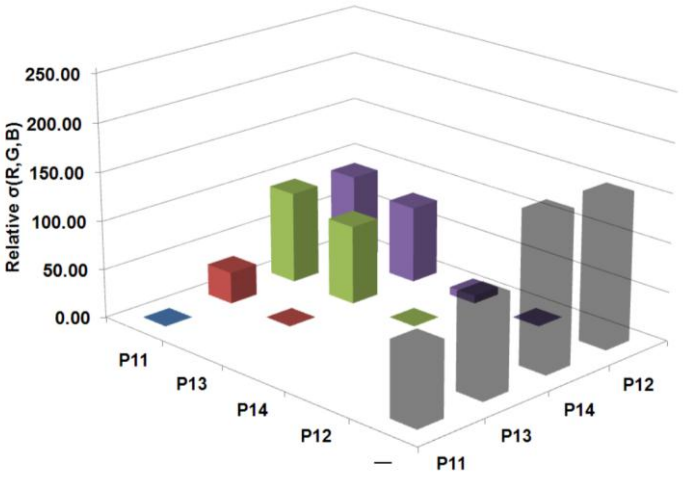

Figure S7. Correlation of σ' values for R/G/B values of phenols **P11**–**P14** using cruciform **1** as the sole sensor. Correlation with the blank sample is given in the row of semi-transparent bars marked with "—".

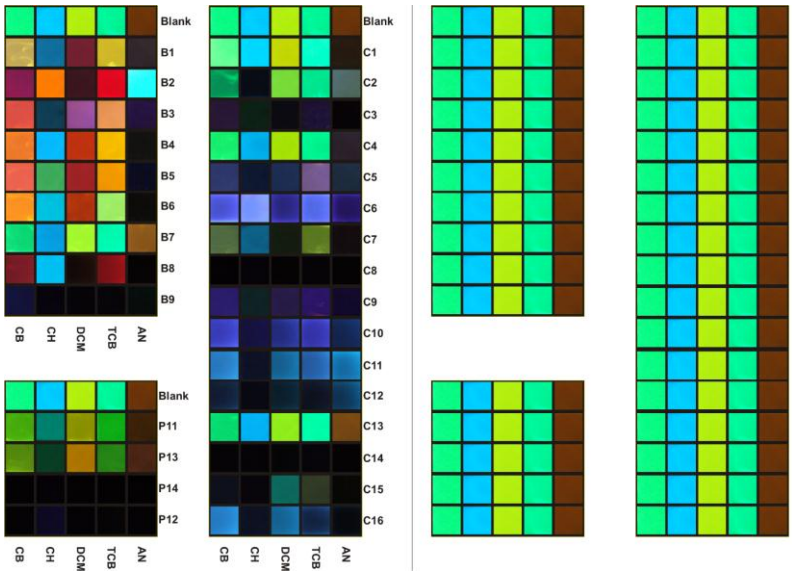

Figure S8. Panel on the right contains segments of photographs of emission colors of analytes studied in this work, while panel on the left represents a "mask image" in which each row has only cruciform **1**'s emission colors.

ImageJ offers at least three ways of subtracting these two images from each other. One way is to subtract the blank from the sample (ImageJ's command *Process* > *Image Calculator* > *Subtract*), which is mathematically described as $RGB_{residual} = RGB_{sample} - RGB_{blank}$. The graphical outcome of this transformation is shown in Figure S9, left. It is important to note that when the sample solution is darker than the blank (i.e. its RGB values

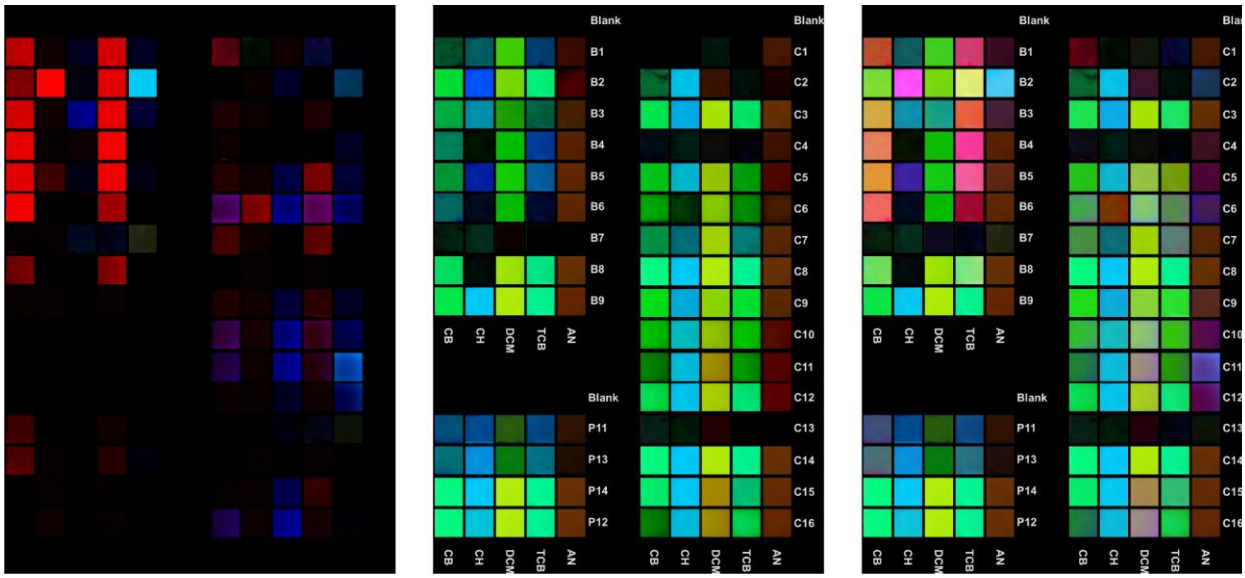

Figure S9. Three possible ways of subtracting the two images shown in Figure S6 from each other, using ImageJ image processing software. On the left, blank image is subtracted from the sample image (using ImageJ *Subtract* command). In the center, *Subtract* command was used to achieve the inverse—subtraction of the sample image from the blank image. Finally, image on the right shows the use of ImageJ's *Difference* command, the outcome of which is independent on the order of image input.

are lower), the corresponding subtraction will produce a negative number—*which will be converted into a zero by ImageJ!* Thus, if a sample completely quenches the fluorescence of the cruciform, its residual RGBs will all be zero, even though the emission colors will obviously be very different from those observed for the blank solution of the sensor—as seen for e.g. 3-nitrosalicylic acid. In addition, this means that a different result is obtained if the ordering in subtraction is inverted, and sample is subtracted from the blank ($RGB_{residual} = RGB_{blank} - RGB_{sample}$, shown in Figure S9, center).

The third option is the use of ImageJ's *Process* > *Image Calculator* > *Difference* command, which produces the absolute value of the RGB differences, described as $RGB_{residual} = |RGB_{sample} - RGB_{blank}|$. Now, a negative number is not treated as zero, but is simply converted into its positive counterpart, and the outcome is invariable to the order of subtraction (Figure S9, right).

We suggest this last method (Figure S9, right) as the most appropriate; not only because it produces the largest standard deviation values (since it eliminates all the "fake zeros"), but is also the closest to the statistical treatment of standard deviations, calculated as:

$$\sigma_{\boldsymbol{C1@AN}} = \sqrt{\frac{(R_{\boldsymbol{C1}}^{AN} - R_{\boldsymbol{1}}^{AN})^2 + (G_{\boldsymbol{C1}}^{AN} - G_{\boldsymbol{1}}^{AN})^2 + (B_{\boldsymbol{C1}}^{AN} - B_{\boldsymbol{1}}^{AN})^2}{3}}.$$

Because of the squaring of the RGB differences, statistical analysis *de facto* deals with the absolute values of those differences.

Fluorescence Titrations of Selected Analytes

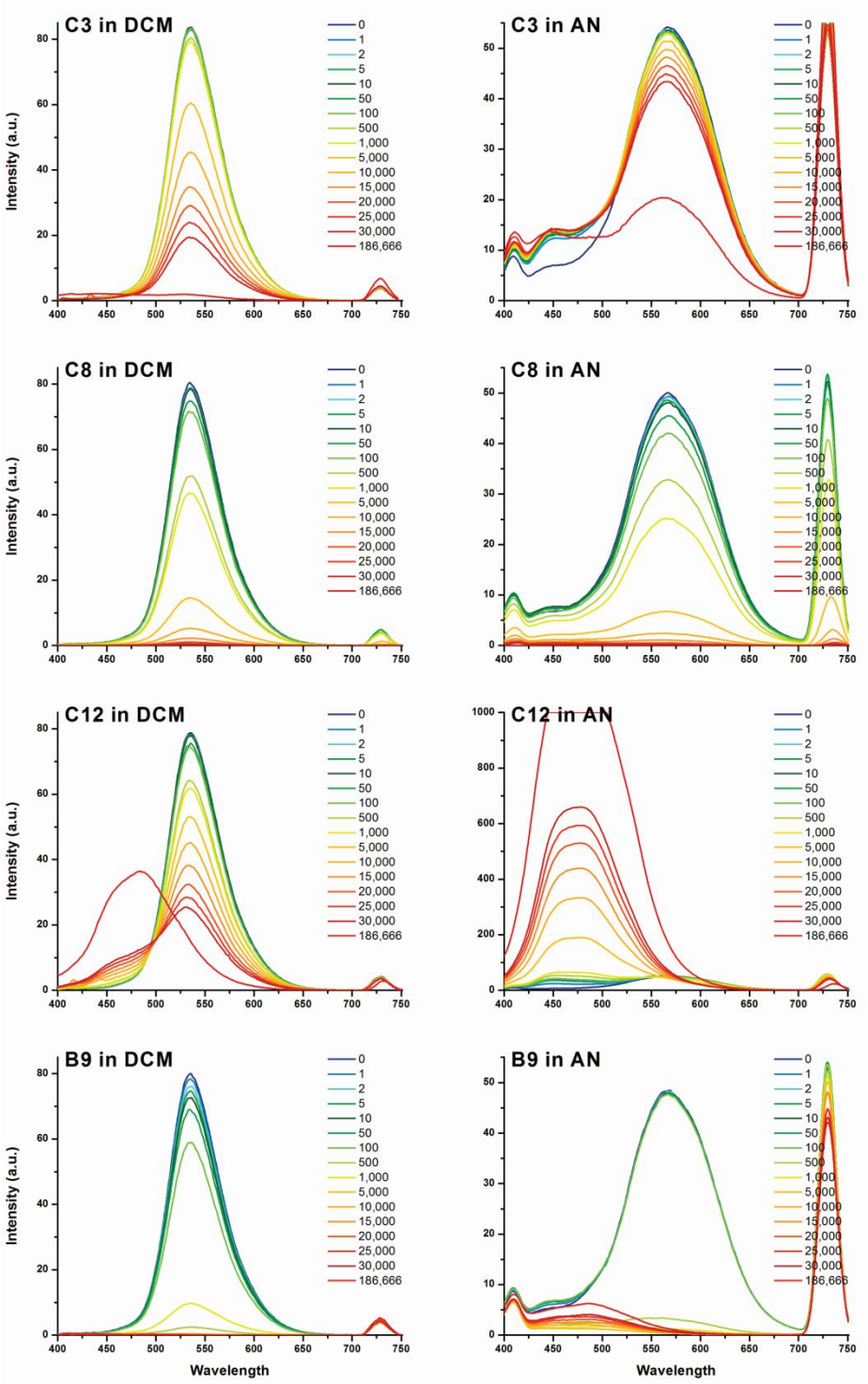

Figure S10. Fluorescence spectral titrations of cruciform **1** with analytes **C3**, **C8**, **C12**, and **B9** (from top to bottom), in DCM (left) and AN (right). Acids **C8** and **B9** quench **1**'s fluorescence quickly; compound **C3** partially quenches it, while **C12** causes a blue emission shift at high analyte concentrations. Numbers on the right-hand side of each graph indicate the number of equivalents of the analyte.

References

(S1) Lim, J.; Albright, T. A.; Martin, B. R.; Miljanić, O. Š., submitted.

(S2) Colour Contrast Analyzer is a downloadable freeware program: http://www.visionaustralia.org.au/info.aspx?page=628.

(S3) ImageJ can be freely downloaded from: http://rsbweb.nih.gov/ij/download.html.