Supporting Information

A fluorescent alcohol biosensor using a simple microPAD based detection scheme

P.D. Thungon[1,2], H. Wang[1], S. Vagin[3], C. Van Dyck[4], S. Goswami[2], B. Rieger[3], A. Meldrum[1]

[1] Department of Physics, University of Alberta, Edmonton, AB, T6G 2E1, Canada

[2] Indian Institute of Technology Guwahati, Guwahati, Assam, 781039, India

[3] Wacker Chair of Macromolecular Chemistry, Technical University of Munich, Lichtenbergstraße 4, 85747 Garching bei München, Germany

[4] Department of Physics, University of Mons, 20 Place du Parc, 7000 Mons, Belgium

Synthetic details and NMR analysis

All solvents and reagents used in the synthesis were purchased from commercial sources and were applied without additional purification, unless stated otherwise. ^{1}H NMR spectra were measured on Bruker AV-III 300 MHz and Bruker AV-III HD 500 MHz spectrometers.

6-(4-methoxyphenoxy)-1-hexanoic acid (1)

1 g, (8 mmol) of p-methoxyphenol is dissolved in 10 ml ethanol and degassed. 1.16 g of KOH pellets with 85% content was added under argon and stirred until complete dissolution. Then 2 g (10 mmol) of 6-bromohexanoic acid was added under argon in one shot. Formation of a solid cake is observed upon stirring. The reaction flask was closed with an overpressure valve and the mixture was heated to 80°C. Upon warming up, the solid dissolved forming a clear solution, which subsequently turned opaque due to the formation of a new precipitate. The stirring at 80°C was continued for 72 hours. After cooling, the mixture was transferred into a beaker with 100 ml of water, stirred until complete dissolution of the solid, then acidified with concentrated HCl to pH=2 and allowed to stand closed overnight. The formed precipitate was filtered off on a Büchner funnel, washed thoroughly with water and dried in a petri dish on air overnight. Yield 1.6 g (83%) of 6-(4-methoxyphenoxy)-1-hexanoic acid. The product was used for further reactions without additional purification. Alternatively, it can be additionally purified by recrystallization with hot filtration using cyclohexane.

^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 6.83 (s, 4H), 3.91 (t, ^{3}J = 6.4 Hz, 2H), 3.77 (s, 3H), 2.40 (t, ^{3}J = 7.4 Hz, 2H), 1.84-1.64 (m, 4H), 1.58-1.46 (m, 2H).

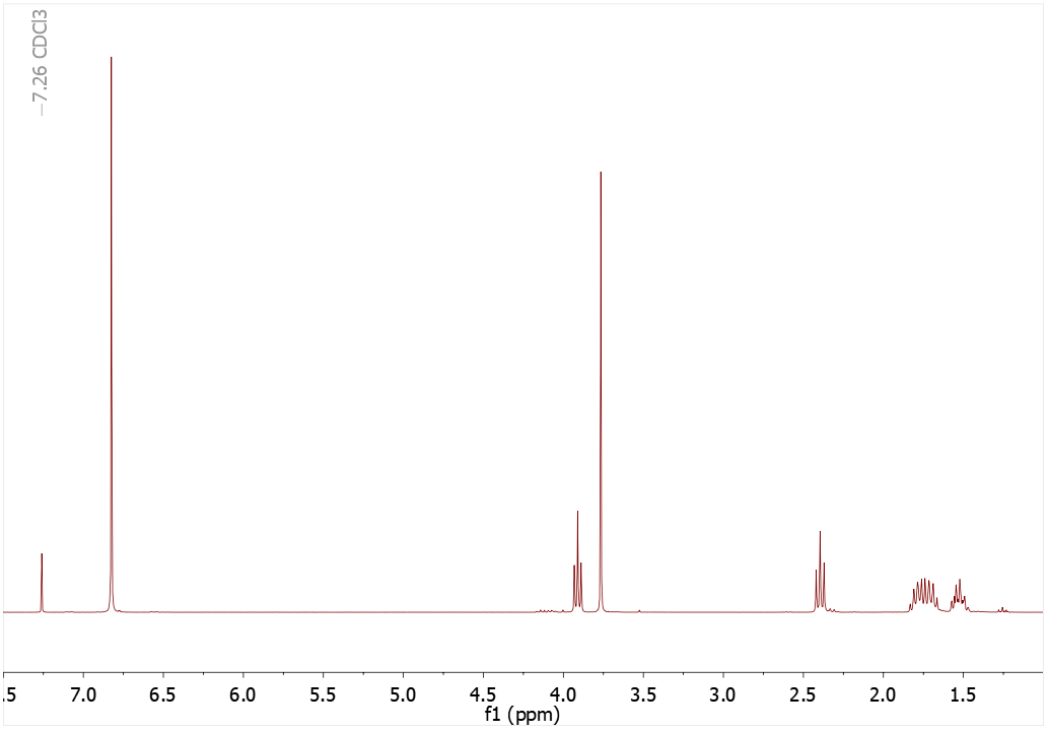

Fig. S1 (a). ^{1}H NMR (300 MHz, $CDCl_3$) spectrum of 6-(4-methoxyphenoxy)-1-hexanoic acid (**1**)

6-(2,5-bis-(bromomethyl)-4-methoxyphenoxy)-1-hexanoic acid (2)

2.38 g (10 mmol) of 6-(4-methoxyphenoxy)-1-hexanoic acid was dissolved in 25 ml glacial acetic acid. 0.66 g (22 mmol) paraformaldehyde and 4.5 ml HBr in acetic acid (32%) were subsequently added. The flask was closed with an overpressure valve connected to a wash bottle with aqueous sodium carbonate solution. The mixture was heated to 60°C and stirred overnight. Thick precipitate is formed during the reaction or after cooling to room temperature. If no precipitate is observed, the metastable oversaturated solution of the product was exposed to air by removing the overpressure valve and stirring for a few minutes. After the precipitation has started, the mixture was allowed to stand closed for several hours and then filtered on a Büchner funnel. The solid product was washed with a small portion of cold acetic acid (ca. 10 ml) followed by extensive washing with water. The product was transferred into petri dish and dried on air overnight, yielding 2.8 g (66%) of 6-(2,5-bis-(bromomethyl)-4-methoxyphenoxy)-1-hexanoic acid. Further purification can be performed by a quick washing of the product with small amounts of cold methanol.

^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 6.79, 6.77 (2s, 2H), 4.44, 4.43 (2s, 4H), 3.91 (t, ^{3}J = 6.3 Hz, 2H), 3.77 (s, 3H), 2.25 (t, ^{3}J = 7.3 Hz, 2H), 1.81-1.69 (m, 2H), 1.68-1.55 (m, 2H), 1.54-1.41 (m, 2H). (addition of a droplet of methanol maybe necessary to achieve a complete dissolution of the sample).

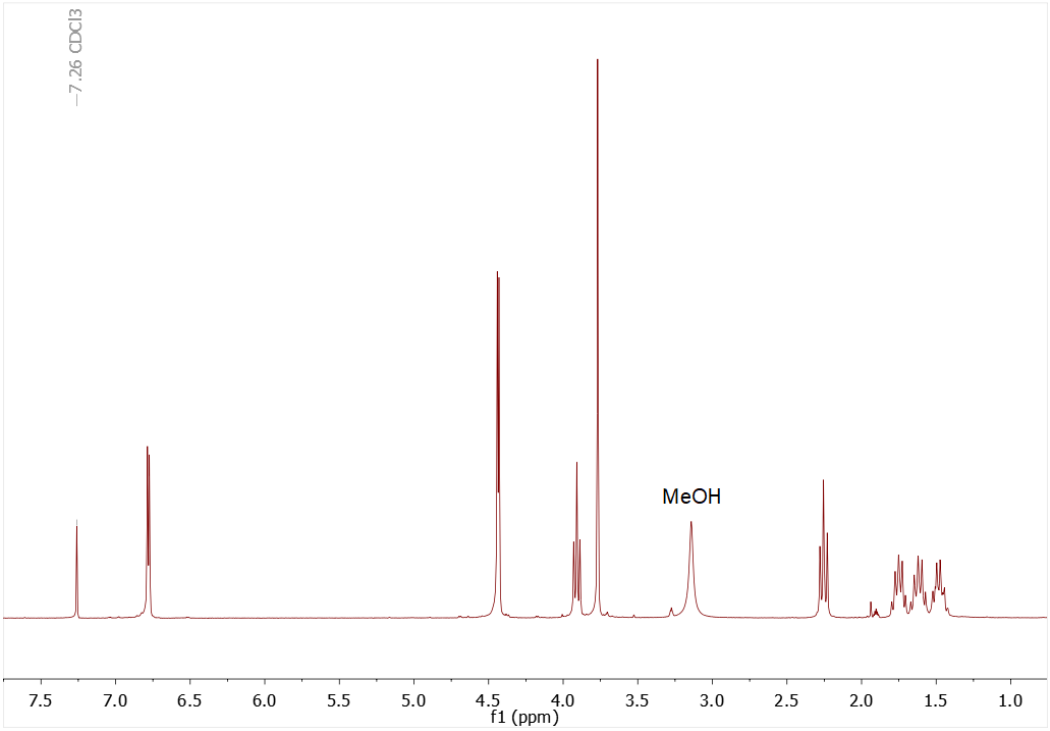

Fig. S1 (b). ^{1}H NMR (300 MHz, $CDCl_3$) spectrum of 6-(2,5-bis-(bromomethyl)-4-methoxyphenoxy)-1-hexanoic acid (**2**)

Ethyl 6-(2,5-bis((diethoxyphosphoryl)-methyl)-4-methoxyphenoxy)hexanoate (3)

1g (2.36 mmol) of 6-(2,5-bis-(bromomethyl)-4-methoxyphenoxy)-1-hexanoic acid was dissolved in 25 ml triethylphosphite and degassed. The flask was closed with an overpressure valve and the reaction solution was stirred at 150 °C for 72 hours. After cooling, the excess of triethylphosphite and diethyl ethylphosphonate (formed as a by-product in this reaction) were removed under vacuum at elevated temperature. The viscous-liquid residue was vigorously stirred with 20 ml water and taken up into ether. The aqueous phase was extracted additionally two times, the organic phase combined and washed with 20 ml water. The organic phase was dried over magnesium sulfate and the ether was removed, yielding 1.16 g (87%) of liquid ethyl 6-(2,5-bis((diethoxyphosphoryl)methyl)-4-methoxyphenoxy)hexanoate, which was used for conversion without additional purification.

^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 6.92-6.87 (m, 2H), 4.12 (q, ^{3}J = 7.1 Hz, 2H), 4.06-3.95 (m, 8H), 3.91 (t, ^{3}J = 6.4 Hz, 2H), 3.78 (s, 3H), 3.27-3.13 (m, 4H), 2.32 (t, ^{3}J = 7.5 Hz, 2H), 1.84-1.62 (m, 4H), 1.54-1.41 (m, 2H), 1.28-1.18 (m, 15H).

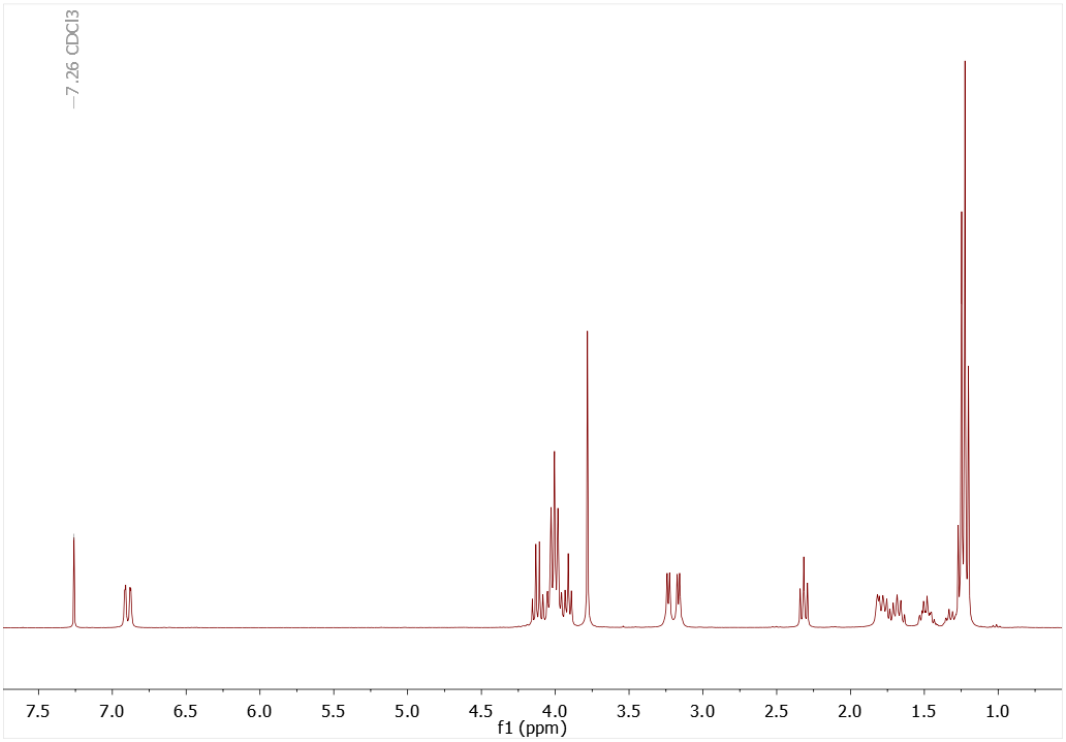

Fig. S1 (c). ^{1}H NMR (300 MHz, $CDCl_3$) spectrum of ethyl 6-(2,5-bis((diethoxyphosphoryl)-methyl)-4-methoxyphenoxy)hexanoate (**3**)

Sodium 6-(4-methoxy-2,5-bis((E)-2-(pyridin-4-yl)vinyl)phenoxy)hexanoate (c-P4VB, 4)

500 mg (0.88 mmol) ethyl 6-(2,5-bis((diethoxyphosphoryl)methyl)-4-methoxyphenoxy)hexanoate **3** was dissolved in 20 ml of dry t-BuOH. Then, 450 mg KOtBu (4 mmol) was added and the solution turned red. After stirring for 10 minutes at room temperature, 240 mg of 4-pyridinecarbaldehyde (2.2 mmol) was added slowly, the reaction mixture turned to dark brownish-green and became slightly warmer. After stirring for 30 min at room temperature, it was heated to 60°C and left overnight. After this, all the volatilities were removed under vacuum. The brown-orange residue was mixed vigorously with aqueous 2M NaOH solution (40 ml) until formation of a fine suspension and heated to 90°C for approx. 10 minutes. After cooling, it was centrifuged, the red solution discarded and the yellow-orange precipitate re-suspended in 5 ml of 2M NaOH. This was heated for 10 minutes at 90°C, cooled and centrifuged, and such washing procedure was repeated once again. Then the precipitate was re-suspended in 2 ml of water followed by centrifugation and such washing was repeated one more time. After drying *in vacuo*, the yellow solid was re-suspended in dry THF (50 ml) and stirred until formation of fine homogeneous suspension. The solid was collected by suction filtration and dried *in vacuo* giving 155 mg (40%) of c-P4VB (product **4**) typically with ca. 98% purity according to ^{1}H NMR (see below).

^{1}H NMR (500 MHz, methanol-d4, 20 mg/ml), δ (ppm): 8.49-8.43 (m, 4H), 7.77-7.69 (m, 2H), 7.56-7.51 (m, 4H), 7.30-7.18 (m, 4H), 4.10 (q, ^{3}J = 6.5 Hz, 2H), 3.94 (s, 3H), 2.24 (t, ^{3}J = 7.4 Hz, 2H), 1.91 (p, ^{3}J = 6.7 Hz, 2H), 1.81-1.68 (m, 2H), 1.67-1.54 (m, 2H).

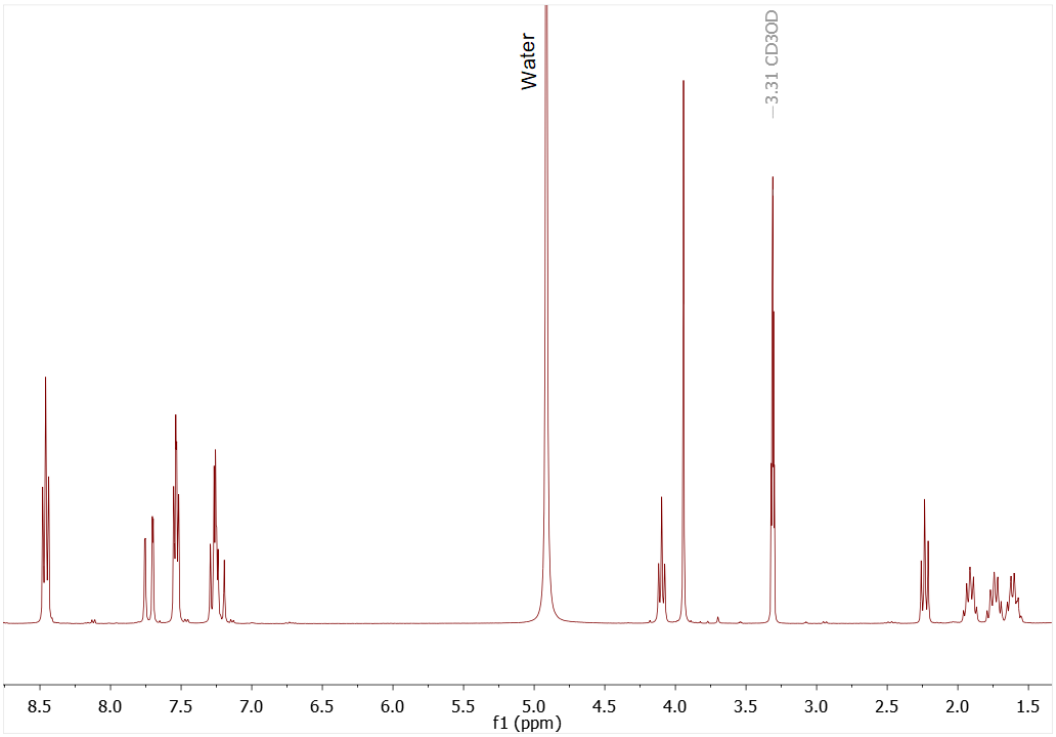

Fig. S1 (d). ^{1}H NMR (300 MHz, methanol-d4) spectrum of sodium 6-(4-methoxy-2,5-bis((E)-2-(pyridin-4-yl)vinyl)phenoxy)hexanoate (c-P4VB, **4**)

The chemical shifts of the protons in the aromatic region are sensitive to the concentration and to the water content in deuterated methanol. Due to the unsymmetrical substitution pattern at the central benzene ring of c-P4VB, the signals of positionally-equivalent protons in two pyridine groups as well as in two vinylene groups are spectrally not equivalent, giving rise to a somewhat more complicated pattern as compared for example with Np-P4VB [S. Lane, S. Vagin, H. Wang, W. R. Heinz, W. Morrish, Y. Zhao, B. Rieger, A. Meldrum, *Light - Sci. Appl.* **2018**, *7*, 101]. This becomes clear when spectra measured on spectrometers with different field strength are compared (see below).

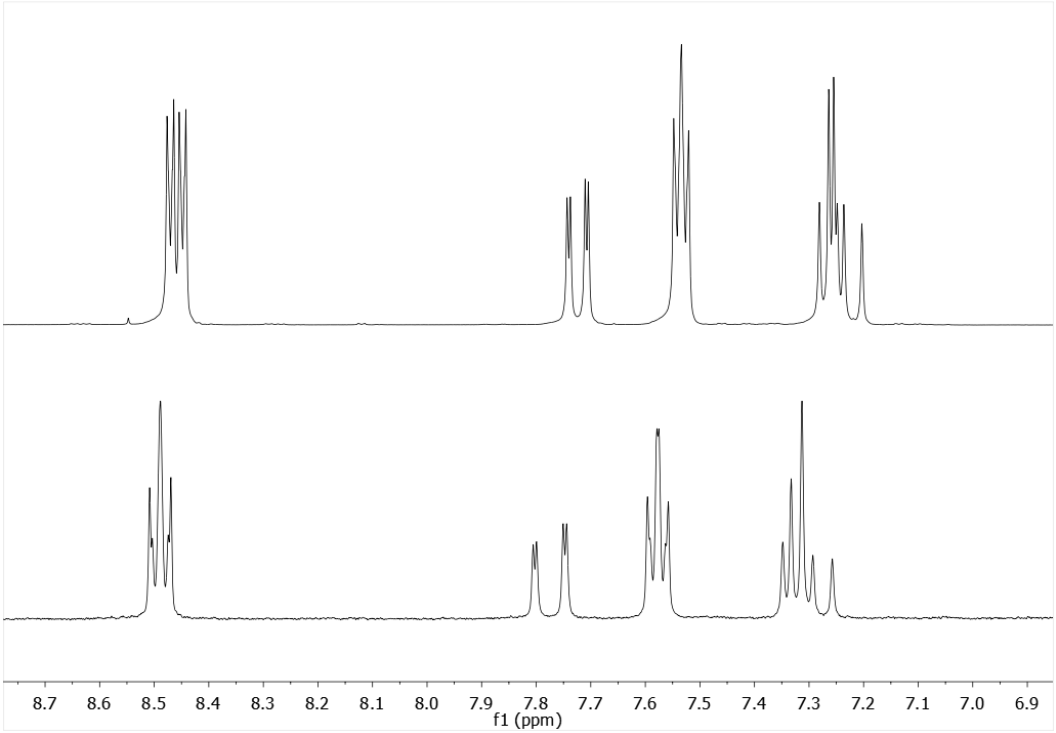

Fig. S1 (e). Signals of protons of c-P4VB in the aromatic region at 500 MHz (top, more concentrated) and 300 MHz (bottom, less concentrated), referenced to the signal of deuterated methanol.

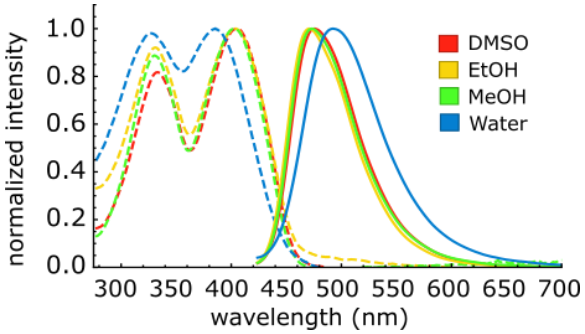

Fig S2. Normalized absorption (dashed lines) and PL (solid lines) spectra for 0.02 mM c-P4VB in dimethyl sulfoxide (DMSO), ethanol (EtOH), methanol (MeOH) and water.

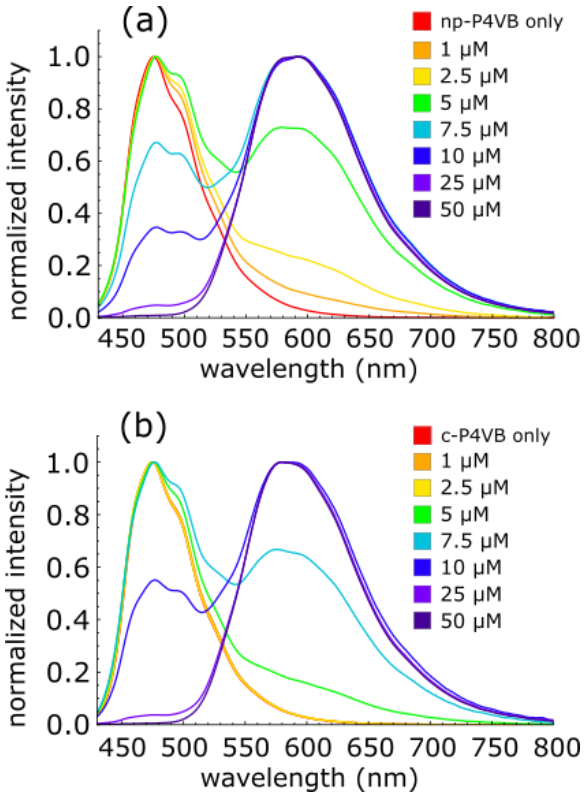

Fig S3. Fluorescence spectra for (a) np-P4VB and (b) c-P4VB at different concentration of HCl (all intensities are normalized; 0.2 mM c-P4VB in ethanol)

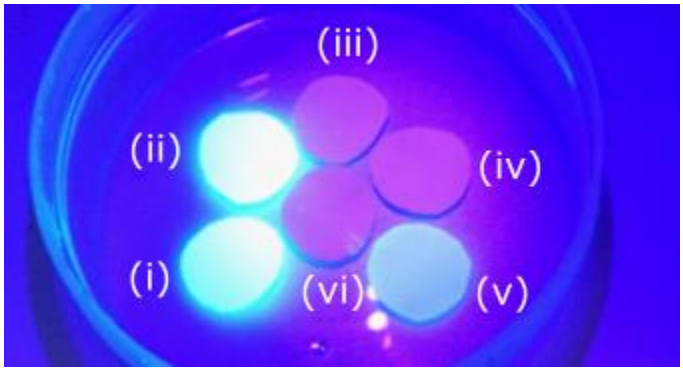

Fig. S4. Paper discs : (i) infused with c-P4VB solution, not washed; (ii) infused with c-P4VB solution, washed with water; (iii) infused with np-P4VB (ethanol solution of high dye concentration) and washed with methanol; (iv) infused with np-P4VB (ethanol solution of low dye concentration) and washed with methanol; (v) infused with c-P4VB solution and washed according to our protocol with water and then methanol followed by sonication; (vi) in the middle – a blank sample.

Fig. S5. Solution of c-P4VB in Millipore Direct-Q™ water (18.2 MΩ) after standing for three days without being tightly closed: This sample evolved a red precipitate as shown (left cuvette). In the right cuvette we show an identical sample saturated with CO_2 by gentle flow of the gas through the solution and stored closed for 3 days, in which already we observe some evidence of precipitation.

The addition of water to c-P4VB in alcohol solutions turned the solution from yellow to light orange, corresponding to the appearance of a protonation-related absorption feature near 500 nm, especially for low dye concentrations. This effect was negligible for fresh 18 MΩ or Millipore water (MQ) but was sometimes noticeable for old MQ or tap water. A degassing treatment could revert the visible color back to yellow and removed the 500-nm absorption band (Fig. S6). This is attributed to the presence of CO_2 (and thus carbonic acid) in water that was left open to the atmosphere. The equilibrium pH of water exposed to atmospheric CO_2 can be as low as ~5.65 due to the presence of carbonic acid; thus, care must be taken to minimize exposure of c-P4VB solutions to water with high CO_2 concentration by using fresh or degassed MQ.

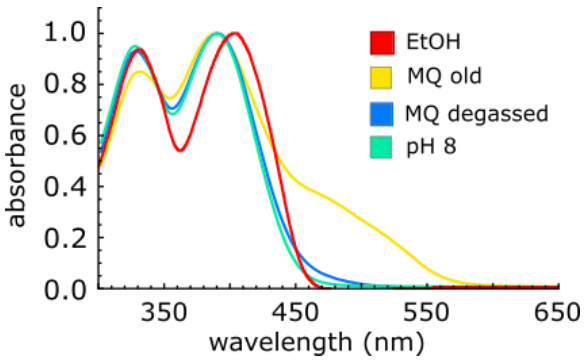

Fig. S6. Absorption spectra for c-P4VB (0.02 mM) dissolved in EtOH, "old" MQ water (left standing in air for several days), the same MQ water after degassing, and in pH8 buffer solution.

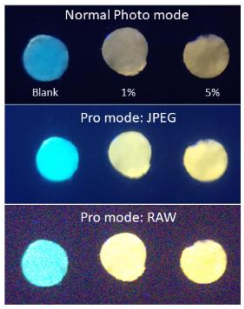

Figure S7: Comparison of image file formats taken using a OnePlus smartphone with treated c-P4VB paper under UV lamp. The c-P4VB paper was treated with different concentration of H_2O_2 and the blank is just c-P4VB paper. Top: auto mode; middle: Pro mode with selected settings to achieve high brightness and contrast; bottom: Pro mode with raw image format.

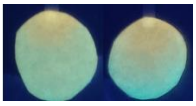

Figure S8: Comparison of the Hue signals between EtOH (left) and MeOH (right) at 0.1% aqueous.

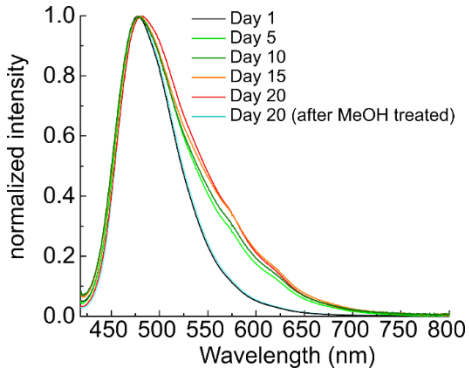

Figure S9: Normalized fluorescence intensity of c-P4VB at different storage time and its reversibility with MeOH treatment. The blue line (Day 20 after MeOH treatment) almost exactly overlies the black line (Day 1).

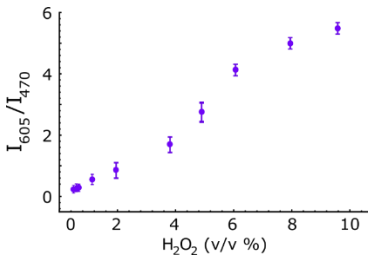

Figure S10: Calibration curve of the paper sensor for different H_2O_2 concentration in aqueous solution, up to high concentrations.