Supplementary materials:

New ratiometric optical oxygen and pH dual sensors with three emission colors for measuring photosynthetic activity in Cyanobacteria

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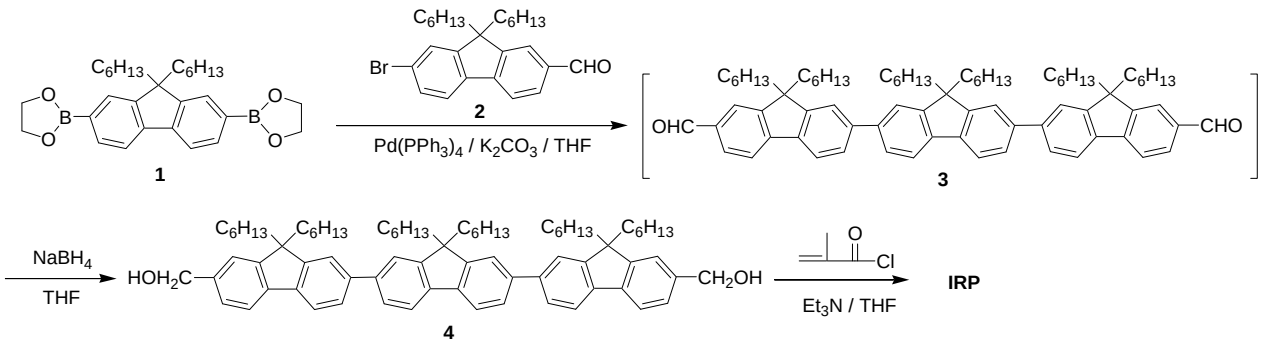
Synthesis of **4** (S-Scheme 1). A mixture of 1.4 g of compound **1** (2.79 mmol) from Sigma-Aldrich, 3.0 g of compound **2** [S1] (6.83 mmol), and 40 mg of $Pd(PPh_3)_4$ was suspended in 30 mL of THF and 10 mL of 2M K_2CO_3 aqueous solution. The mixture was heated at 80 °C under nitrogen for 16 hours. After pouring the reaction mixture into water, the organic material (intermediate **3**) was extracted into CH_2Cl_2 and was used without purification. The raw material **3** was dissolved in 20 mL THF. 0.2 g of $NaBH_4$ was added into the THF solutions. The mixture was stirred at room temperature for 6 hours. After adding the THF with 20 mL of cold water, the organic materials were extracted into CH_2Cl_2. After removing the CH_2Cl_2, the product was purified by column chromatography and then crystallized from methanol to get the compound **4**. Yield: 40%. ^{1}H NMR ($CDCl_3$, δ, ppm): 7.79 (m, 6H), 7.66 (m, 8H), 7.33 (m, 4H), 4.79 (s, 4H), 2.05 (m, 12H), 1.07 (m, 36H), 0.74 (m, 30H). ^{13}C NMR ($CDCl_3$, δ, ppm): 151.76, 151.61, 151.53, 140.53, 140.46, 139.97, 139.75, 126.12, 125.86, 125.59, 121.49, 119.89, 119.94, 119.74, 65.84, 55.29, 55.17, 40.34, 31.46, 31.42, 29.67, 23.79, 22.56, 22.52, 13.99. MALDI-Mass: $C_{77}H_{102}O_2$ Calc. 1058.79, found: 1058.84.

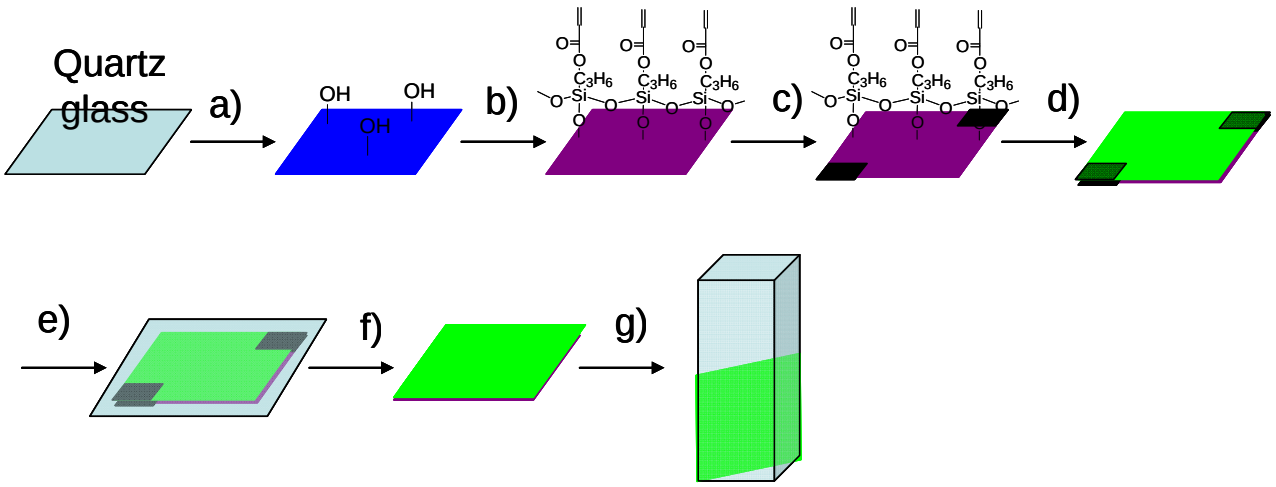
Synthesis of **IRP**. 500 mg of methacryloyl chloride (5 mmol) in 1 mL THF was added to a solution of 300 mg of compound **3** (0.28 mmol) in 10 mL of anhydrous THF with 1 mL of Et_3N at 0 - 5°C. The mixture was warmed to room temperature and the reaction mixture was stirred at room temperature for overnight. The mixture was poured into 100 mL of water. The product was extracted into 100 mL of CH_2Cl_2. After the CH_2Cl_2 was removed the product was crystallized from methanol to obtain 200 mg of product of **IRP**. Yield: 59%. ^{1}H NMR (400 MHz, $CDCl_3$): 7.79 (4H, m), 7.65 (10H, m), 7.61 (4H, m), 6.17 (s, 2H), 5.59 (s, 2H), 5.27 (s, 4H), 2.01 & 1.98

(18H, m & s), 1.07 (m, 36H), 0.75 (m, 30H). ^{13}C NMR ($CDCl_3$, δ, ppm): 167.31, 151.76, 151.71, 151.371, 140.87, 140.67, 140.41, 139.99, 136.36, 134.77, 127.03, 126.09, 125.67, 122.83, 121.44, 119.99, 119.69, 66.89, 55.29, 55.15, 40.23, 31.42, 29.61, 23.77, 22.51, 18.35, 13.99. MALDI-Mass: $C_{85}H_{110}O_4$ Calc. 1194.84, found: 1194.99.

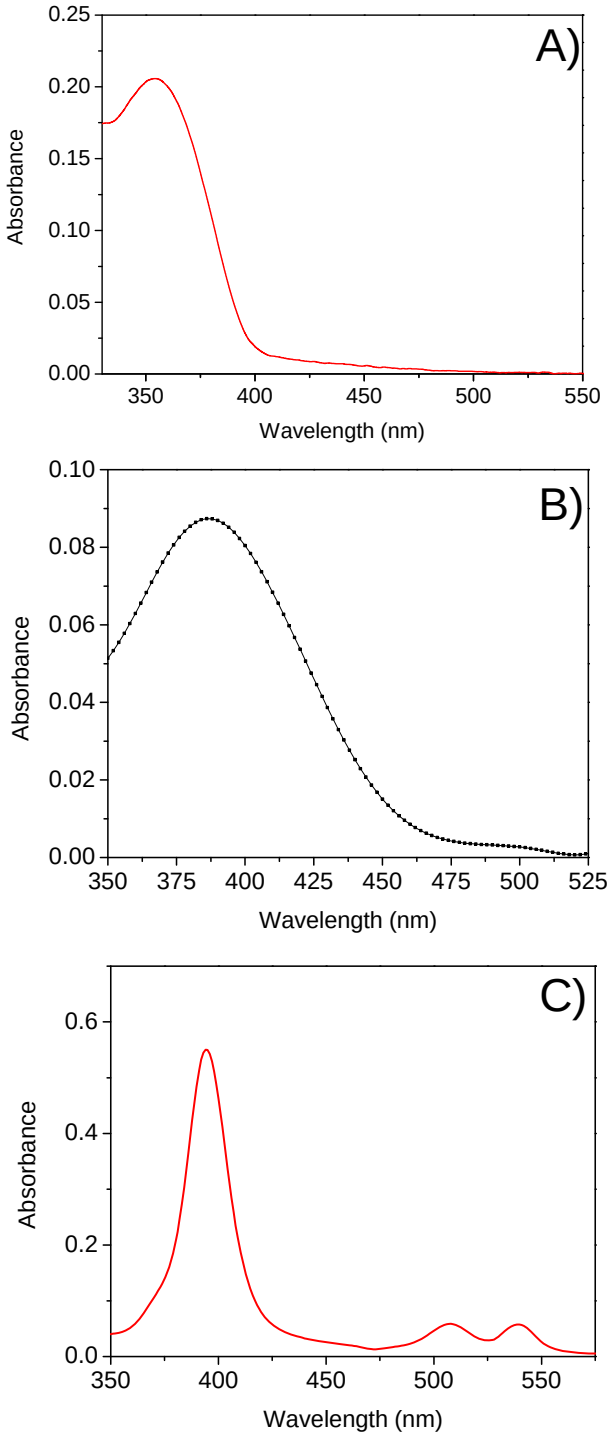
[S1]: Y. Q. Tian, C. -Y. Chen, C. -C. Yang, A. C. Young, S. -H. Jang, W. -C. Chen, A. K. -Y. Jen, *Chem. Mater*., 2008, **20**, 1977-1987.


C6H13 C6H13
Br
CHO
2
1
Pd(PPh3)4 / K2CO3 / THF
OHC
CHO
3
NaBH4
THF
HOH2C
CH2OH
4
O
Cl
Et3N / THF
IRP

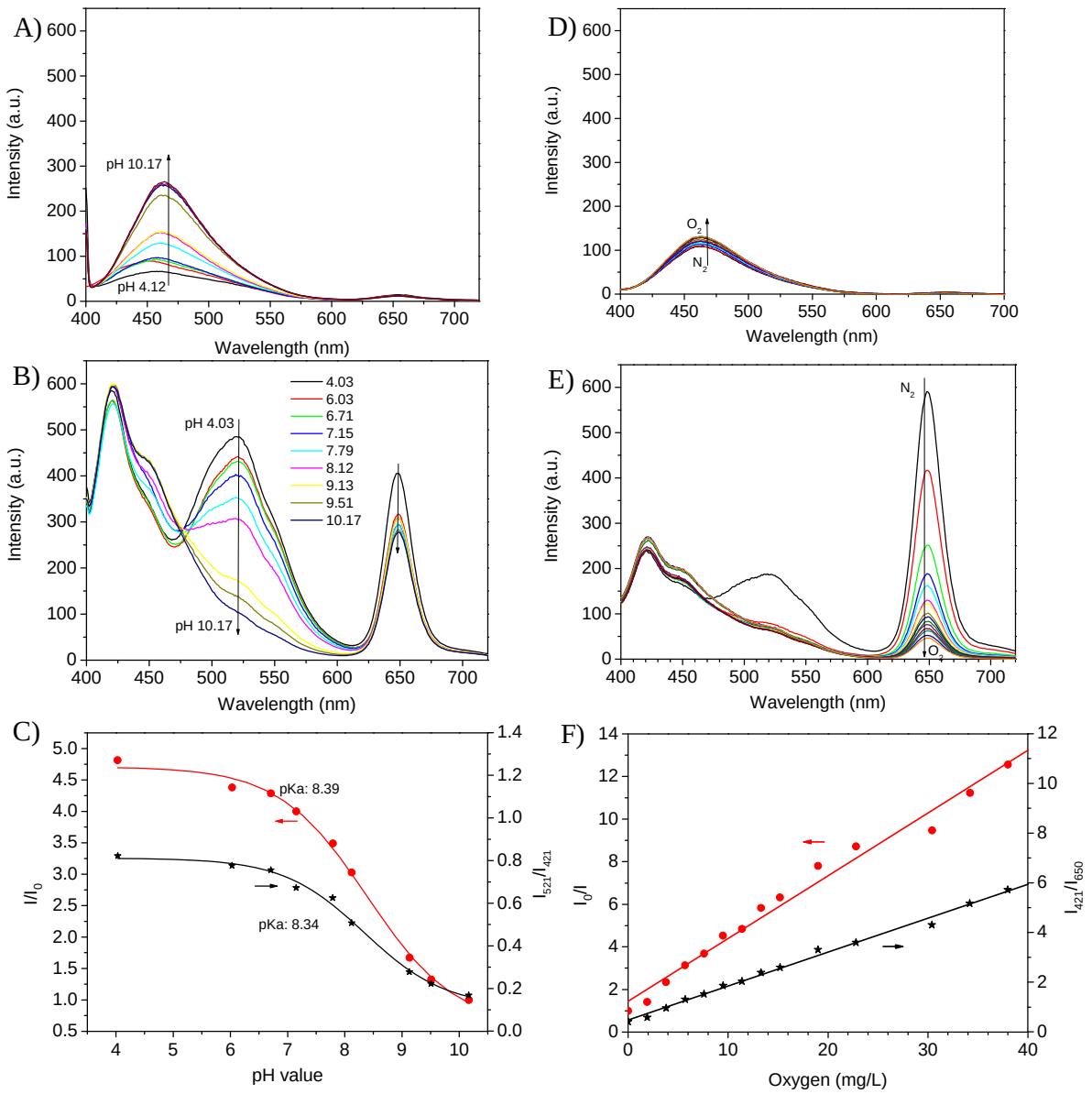
S-Scheme 1



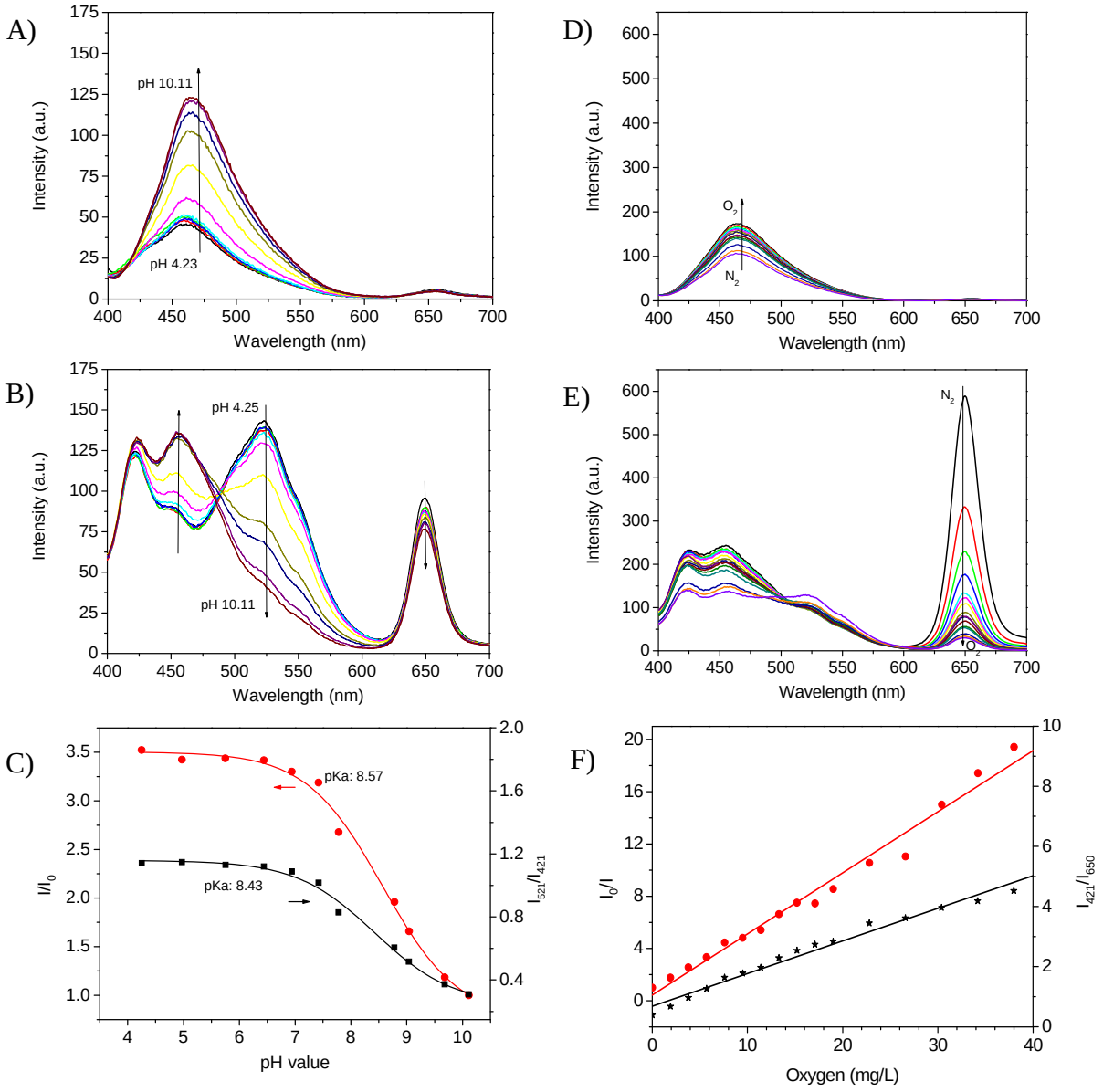
S-Figure 1. Schematic illustration of the preparation of sensing membranes. a) oxygen plasma treatment to generate active hydroxyl groups; b) vapor deposition of thin TMSPA layer; c) 25-μm tape used to control membrane thickness; d) sensor solution dispensed onto modified quartz surface; e) solution covered with a cover glass and polymerized at 80°C for 1.5 hours; f) cover glass and tape removed; film rinsed using methanol and double-distilled water; and g) sensing membrane on quartz substrate immersed into liquid in cuvette for fluorescence measurements.



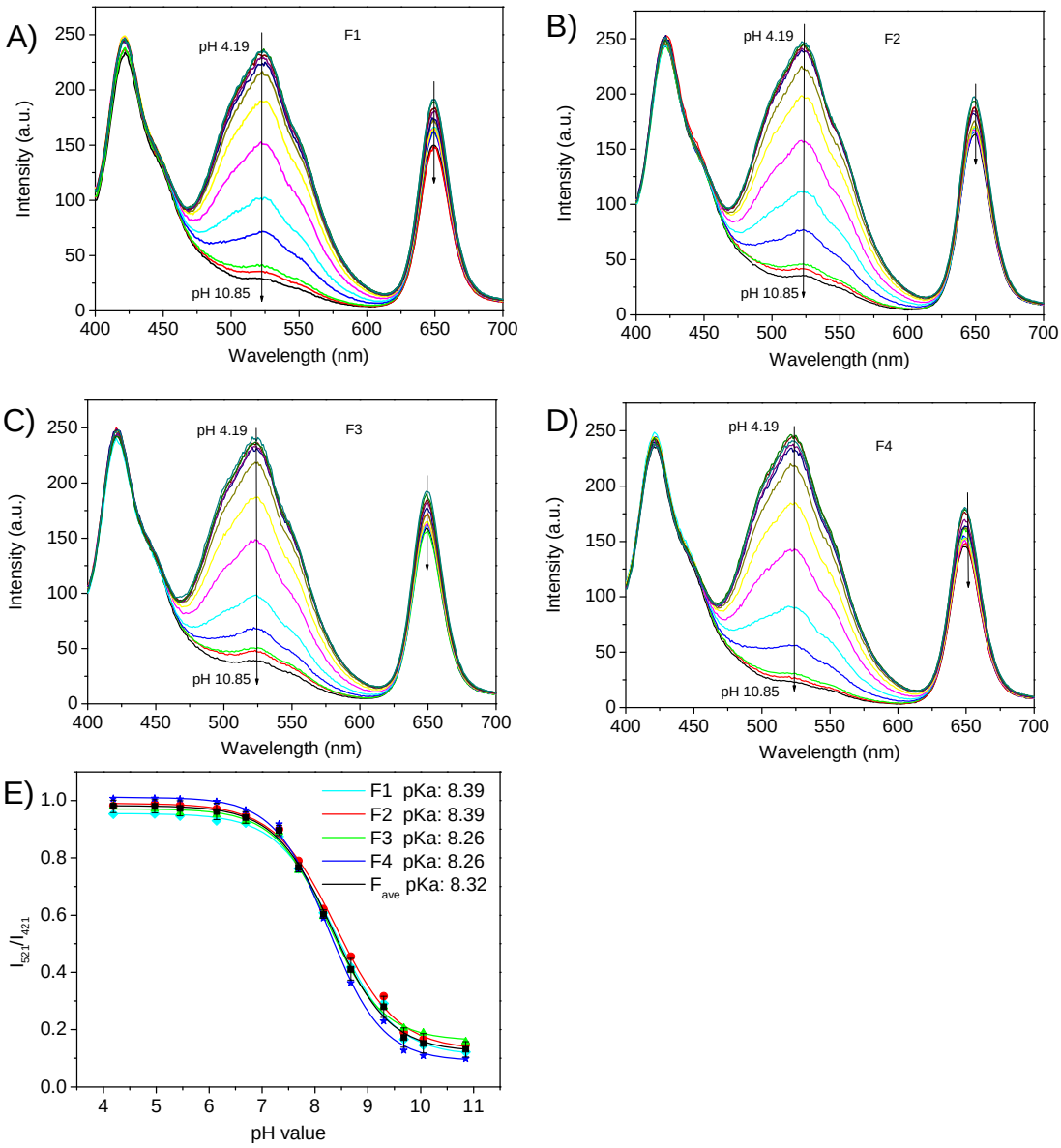
S-Figure 2. Absorbance spectra of the individual **IRP** (A), **pHS** (B), and **OS** (C) in their PHEMA-*co*-PAM thin films.



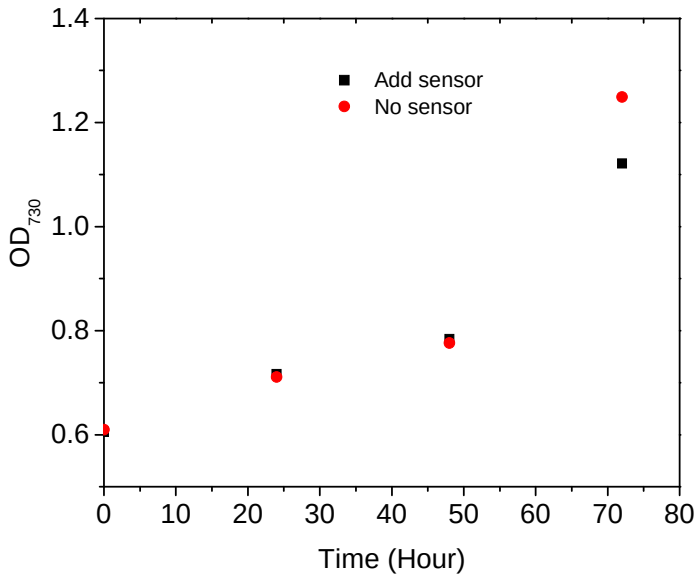
S-Figure 3. pH dependent emission spectra of cyanobacteria (OD_{730} of 0.75) (A); fluorescence spectra of the sensing film with the cyanobacteria at different pH values (B); pK_a values calculated using the **pHS**'s emission intensities at 521 nm and the ratiometric intensities ratios at 521 nm and 421 nm as described in the text (C); dissolved oxygen dependent emission spectra of cyanobacteria (OD_{730} of 0.75) (D); fluorescence spectra of the sensing film with the cyanobacteria at different dissolved oxygen concentrations (E); Stern-Volmer fittings using the **OS**'s emission intensities at 650 nm and the ratiometric intensities ratios at 650 nm and 421 nm as described in the text (F).



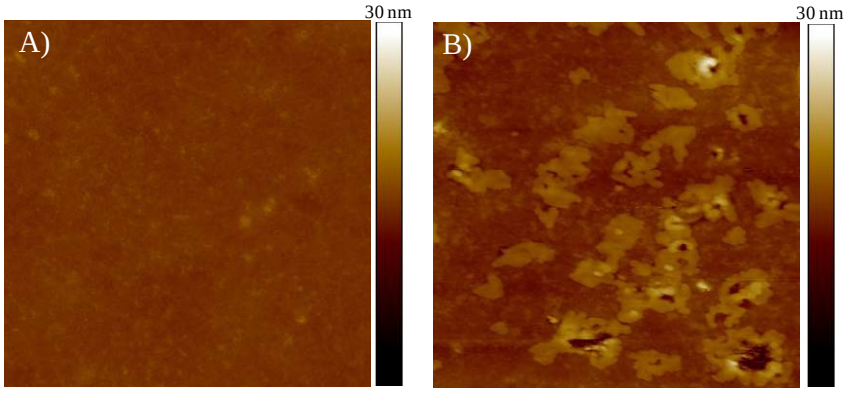
S-Figure 4. pH dependent emission spectra of cyanobacteria (OD_{730} of 1.50) (A); fluorescence spectra of the sensing film with the cyanobacteria at different pH values (B); pK_a values calculated using the **pHS**'s emission intensities at 521 nm and the ratiometric intensities ratios at 521 nm and 421 nm as described in the text (C); dissolved oxygen dependent emission spectra of cyanobacteria (OD_{730} of 0.75) (D); fluorescence spectra of the sensing film with the cyanobacteria at different dissolved oxygen concentrations (E); Stern-Volmer fittings using the **OS**'s emission intensities at 650 nm and the ratiometric intensities ratios at 650 nm and 421 nm as described in the text (F).



S-Figure 5. pH dependent emission spectra of four individual films (F1- A, F2- B, F3-C, and F4-D) in cyanobacteria (OD_{730} of 0.5). E gives the comparison of the pK_a values of the four films and the average pK_a value for demonstration of the reproducibility of the films.



S-Figure 6. The changes of OD_{730} of cells with and without a sensor film. The sensor film has no obvious toxicity to cells for 48 hours.



S-Figure 7. Surface morphologies of a typical sensing film freshly prepared (A) and after swollen by water (B) measured using atomic force microscopy (AFM). The image area is 1.5 × 1.5 μm. Roughness of the freshly prepare film is 0.46 nm. Roughness of the swollen film is 3.6 nm.