Supplementary materials:

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

Hongguang Lu, Yuguang Jin, Yanqing Tian, Weiwen Zhang, Mark R. Holl, Deirdre R. Meldrum

Center for Biosignatures Discovery Automation, The Biodesign Institute, Arizona State University, PO Box 875801, Tempe, AZ 85287-5801
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(PPh₃)₄ was suspended in 30 mL of THF and 10 mL of 2M K₂CO₃ 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 CH₂Cl₂ and was used without purification. The raw material 3 was dissolved in 20 mL THF. 0.2 g of NaBH₄ 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 CH₂Cl₂. After removing the CH₂Cl₂, the product was purified by column chromatography and then crystallized from methanol to get the compound 4. Yield: 40%. \(^1\)H NMR (CDCl₃, 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). \(^1\)³C NMR (CDCl₃, 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: C₇₇H₁₀₂O₂ 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 Et₃N 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 CH₂Cl₂. After the CH₂Cl₂ was removed the product was crystallized from methanol to obtain 200 mg of product of IRP. Yield: 59%. \(^1\)H NMR (400 MHz, CDCl₃): 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$_3$, $\delta$, 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.

Scheme 1

1. Reaction of 1 with NaBH₄ in THF

2. Reaction of 2 with Pd(PPh₃)₄ / K₂CO₃ / THF

3. Reaction of 3 with Et₃N / THF

4. Conversion of 4 to IRP
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 pH'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\textsubscript{730} of 0.5). E gives the comparison of the pK\textsubscript{a} values of the four films and the average pK\textsubscript{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.