Conjugated Polymer-Based Hybrid Materials for Turn-On Detection of CO₂ in Plant PhotosynthesisHongbo Yuan,^{†,§,||} Yibing Fan,^{‡,||} Chengfen Xing,^{*,‡} Ruimin Niu,[‡] Ran Chai,[†] Yong Zhan,^{*,†} Junjie Qi,[‡] Hailong An,[‡] and Jialiing Xu^{*,§}[†]School of Materials Science and Engineering, Hebei University of Technology, Tianjin 300401, P.R. China[‡]Institute of Biophysics, Hebei University of Technology, Tianjin 300401, P.R. China[§]Institute for Molecules and Materials, Radboud University, Heyendaalseweg 135, 6525 AJ Nijmegen, The Netherlands

S Supporting Information

ABSTRACT: Detection of carbon dioxide (CO₂) is of fundamental importance in diverse applications ranging from environmental analysis to agricultural production. In this work, a hybrid probe based on guanidinium-pendent oligofluorene (G-OF) and water-soluble conjugated polythiophene (PTP) has been developed for the turn on detection of CO₂ with low background signal, taking advantage of the efficient fluorescence quenching of the tight aggregate of G-OF/PTP. In the presence of CO₂, the electrostatic repulsion between G-OF and PTP can be effectively enhanced through protonation of the side chains, leading to the disaggregation and thus the “turn-on” fluorescence. The strategy allows for the light-up visible detection of CO₂ with high sensitivity. Importantly, this system is capable of sensitively monitoring the concentration changes of CO₂ in the process of the photosynthesis, which represents a concept to monitor the photosynthesis based on water-soluble conjugated polymers.

Carbon dioxide (CO₂) is a greenhouse gas contributing immeasurably to the global climate change and acts as an asphyxiant with the maximal acceptable concentration defined as 3.0%.^{1–3} CO₂ also plays an essential role in plant photosynthesis. A total of 90–95% of the dry weight of plants derives from the assimilation of CO₂ during photosynthesis.^{4,5} In this context, monitoring the photosynthesis of vegetation is not only crucial for raising the productivity of agriculture but also important for the better fixation of CO₂. Therefore, the detection of CO₂, in particular during the process of photosynthesis, is of great significance for global warming monitoring,^{6,7} medical diagnosis,^{8,9} and agricultural production.¹⁰ Optical CO₂ detection methods have exhibited their advantages including convenience, low cost, and fastness, over the traditional analytical methodologies such as near-infrared spectroscopy,¹¹ gas chromatography/mass spectrometry,^{12,13} and electrochemical techniques.¹⁴ For example, several effective fluorescent probes based on aggregate-induced emission (AIE) have been recently developed for monitoring CO₂.^{15–19} Yoon and coauthors realized colorimetric and fluorescent CO₂ detection by applying polydiacetylene in aqueous solution and in the solid state.²⁰ However, most of the established approaches have relatively high background fluorescence signal or require additional accessory molecules.²⁰ Moreover, to the best of our knowledge, very few examples of optical sensors have been reported to detect the changes of CO₂ in the process of photosynthesis, which is highly desirable.

Water-soluble conjugated polyelectrolytes are characterized by their delocalized π -electronic backbones and extraordinary light-harvesting capacities, exhibiting a signal amplification effect.^{21–24} In the past few years, water-soluble conjugated polymers have been widely employed as an efficient platform for the detection of small molecules^{25–27} and biomolecules such as DNA,^{28–30} protein,^{31–33} and enzymes,^{34,35} with specificity and high sensitivity. They have been further developed into promising materials for broad biological applications in disease diagnosis,³⁶ drug screening and delivery,^{37,38} cell imaging,³⁹ and antimicrobial susceptibility testing.^{40–42} Inspired by these studies, we illustrate here a fluorescent, visible and “turn-on” detection system for CO₂ in dissolved and gaseous states as well as in plant photosynthesis, by hybridizing the guanidinium-pendent oligofluorene (G-OF) and water-soluble conjugated polymer poly(3-(2'-N,N-dipropionic acid-ethylamino)-2,5-thiophene) (PTP). As illustrated in Scheme 1, PTP is designed to be functionalized with one amino group and two carboxylate groups in each repeat unit, while G-OF has guanidinium groups in the side chains. In the absence of CO₂, the guanidinium group of G-OF is partly protonated and the polymer exhibits positive charges, interacting with anionic PTP by tight electrostatic and π - π

Received: April 15, 2016

Accepted: June 2, 2016

Published: June 2, 2016

Scheme 1. (a) Charge Changes of G-OF and PTP upon Bubbling with CO₂ and (b) Schematic Representation of G-OF/PTP Hybrid Probe for CO₂ Detection in Plant Photosynthesis^b

^aThe charge changes of G-OF and PTP according to the different pK_a values of each side chain, the guanidinium, tertiary amine and carboxyl groups with pK_a values of 13.6, 9.54, and 2.2, respectively.^{43,44} ^bThe fluorescence of both polymers is quenched upon the formation of the tight aggregate of G-OF/PTP but gets efficiently recovered in the presence of CO₂.

stacking interactions, forming micrometer-sized supramolecularly assembled aggregates. These strong supramolecular interactions dramatically quenches (turn-off) the fluorescence of both polymers. When CO₂ is bubbled into the solution, the guanidinium groups of G-OF are completely protonated and the polymers become positively charged. At the same time, both the amino and the carboxyl groups of PTP are protonated, endows the polymer also with positive charges. Thus, the electrostatic repulsion between G-OF and PTP dominates and leads to the break of the G-OF/PTP aggregates, resulting in the fluorescence recovery of G-OF and PTP (turn-on). The system is so sensitive that it can be effectively applied to monitor the concentration changes of CO₂ in the process of plant photosynthesis, which continuously converts CO₂ into carbohydrate by the assimilation of sunlight and H₂O and converts.^{45,46} The fluorescence of G-OF/PTP is gradually quenched as the plant photosynthesis is consuming the CO₂. Furthermore, the CO₂ detection can be visualized directly in view of color changes of G-OF/PTP in aqueous medium under UV light.

EXPERIMENTAL SECTION

Materials and Measurements. G-OF and PTP were synthesized referring to the procedure reported in the literature.^{44,47} All chemicals were purchased from Acros, Aladdin, or Alfa Aesar and used as received if not specially stated. All organic solvent were purchased from Tianjin Guangfu Ltd. CO₂, N₂, and O₂ were certified food grade (99.99% purity) and obtained from Tianjin Lianbo Ltd. All solutions were prepared with precooled Milli-Q water at 4 °C. The fluorescence spectra were taken on a Hitachi F-4500 fluorimeter equipped with a xenon lamp excitation source at 4 °C which was controlled by recirculating chiller F-305

(BÜCHI). Dynamic light scattering (DLS) experiments and ζ potentials were carried out on Nano-S90 (Malvern Instruments, U.K.).

Detection of CO₂ in Water. To 400.0 μL of ddH₂O was added G-OF (0.8 μM) and bubbled with different volumes of CO₂ at a constant flow rate of 1.5 mL/min, and then 875.0 nM (in repeat units) of PTP was added and mixed gently. After 5.0 min of incubation, the fluorescence spectra were measured in quartz cuvettes by applying a Hitachi F-4500 fluorimeter at 4 °C. The excitation wavelength was 378 nm.

Preparation for Different Ratios of CO₂/ N₂ Mixtures. The flow rate of CO₂ and N₂ were controlled by standard flowmeter and mixed in a sampling bag.

Detection of CO₂ by Composite Film. G-OF solution (10.0 μM) was sprayed on the cellulose ester film. After 5.0 min of exposure to a CO₂ atmosphere, the PTP (8.0 μM) was sprayed, and then the images were taken under UV light (λ = 365 nm).

Detection of CO₂ in Photosynthesis. An airtight chamber (500 × 500 × 500 mm³) was designed with an inlet, an outlet, lamps (300 W), and a delivery window. *Zea mays* were grown in vermiculite for 14 days in a greenhouse. We eliminated the air in chamber through the method of vacuum and then filled with CO₂ to 1.0 atm. The 1.0 mL of detection solution contained with G-OF ([G-OF] = 0.8 μM) was put in the chamber after different time of illumination. After 12 h of equilibrium, 875.0 nM (in repeat units) of PTP was added, and the fluorescence spectra were measured.

RESULTS AND DISCUSSION

PTP and G-OF were synthesized referring to the reported procedures.^{44,47} The detection of CO₂ by the G-OF/PTP hybrid probe is based on the efficient fluorescence quenching of both polymers. G-OF emits bright blue light in aqueous solution with the fluorescence spectrum maximized at 420 nm, while PTP emits yellow fluorescence with a maximum at 560 nm. The fluorescence of G-OF ([G-OF] = 0.8 μM) was quenched upon addition of PTP ([PTP] = 0–875.0 nM in repeat units) in water. As shown in Figure 1a, the emission of

Figure 1. (a) Fluorescence spectra of G-OF in water with successive additions of PTP. (b) K_{SV} plot of G-OF in the presence of PTP. [G-OF] = 0.8 μM, [PTP] = 0–875.0 nM (in repeat units). *I* and *I*₀ represent the emission intensity of G-OF at 420 nm in the presence and absence of PTP. Measurements were performed at 4 °C in sterile water. The excitation wavelength was 378 nm.

G-OF was decreased gradually with successive addition of PTP and the fluorescence of G-OF was efficiently quenched by 97.2% in the presence of PTP ([PTP] = 875.0 nM). The quenching efficiency was calculated by measuring the fluorescent changes of G-OF via the Stern–Volmer equation (eq 1):⁴⁸

$$I_0/I = 1 + K_{SV}[Q] \quad (1)$$

The Stern–Volmer constant (K_{SV}) value was deduced to be $1.38 \times 10^7 \text{ M}^{-1}$ from the linear Stern–Volmer plot with low concentrations of PTP (0–25.0 nM) (Figure 1b), indicating the superquenching of G-OF by PTP. The intense electrostatic and π – π stacking interactions between G-OF and PTP were attributed to drive the formation of the tight aggregation of G-OF/PTP complex and to induce the superquenching behavior.

The sensitivity of the assembled G-OF/PTP complex to CO_2 was first examined in the solution state, by bubbling CO_2 into the aqueous solution of G-OF in the presence of PTP and the results are summarized in Figure 2. As shown in Figure 2a,

Figure 2. (a) Fluorescence spectra of G-OF in the presence of PTP with and without CO_2 . (b) Fluorescence intensity of G-OF at 420 nm in the presence of PTP after bubbling with different volumes of CO_2 in water at 4 °C. I_0 and I represent the emission intensity of G-OF at 420 nm in the presence of PTP without and with CO_2 , respectively, and $\Delta I/I_0$ is equal to $(I - I_0)/I_0$. All data were presented as mean values $\pm$ standard deviation of three separate experiments. Error bars represent standard deviations of data from three separate measurements. (c) Fluorescence images of G-OF in the presence of PTP in water with and without CO_2 , under UV light excitation ($\lambda = 365 \text{ nm}$). (d) Emission spectra of G-OF in water with and without CO_2 . (e) ζ Potentials of G-OF and PTP with and without CO_2 in water. (f) Size distribution histograms of G-OF in the presence of PTP with and without CO_2 resulting from dynamic light scattering measurement. $[\text{G-OF}] = 0.8 \mu\text{M}$, $[\text{PTP}] = 875.0 \text{ nM}$ (in repeat units), the bubbling volumes of CO_2 increase from 0 to 30.0 mL at a constant flow rate of 1.5 mL/min. Measurements were performed at 4 °C in sterile water. The excitation wavelength was 378 nm.

the emission intensity of G-OF has been recovered by approximately 20 times after reacting with CO_2 . The recovery rate is dependent on the bubbling volumes of CO_2 . As shown in Figure 2b, the fluorescence intensity of G-OF/PTP at 420 nm increases with the amount of CO_2 bubbled and the detection limit can be as lower as 75.0 μL . Importantly, the detection of CO_2 can be directly visualized through the color changes of G-OF/PTP from dark blue to bright blue under UV irradiation upon bubbling with CO_2 , as shown in the images in

Figure 2c. Control experiments were carried out to check the effect of CO_2 on the emitting behaviors of G-OF and PTP. CO_2 was found to induce very little effect on the emission spectra of G-OF in the absence of PTP (Figure 2d), while the emission of PTP was decreased with the addition of CO_2 (see Figure S3 in the Supporting Information). This is consistent with the working mechanism proposed in Scheme 1, where the net charges of PTP is decreased and the dominating interchain π – π stacking leads to the formation of aggregates and results in the self-quenching of PTP, with the increasing amount of CO_2 .^{49,50}

The ζ potentials provide further evidence for the interactions between G-OF and PTP in the presence and absence of CO_2 (Figure 2e). ζ potentials of G-OF and PTP became more positive upon reaction with CO_2 because the guanidinium groups of G-OF and the amino and carboxylate groups of PTP became completely protonated. Additionally, the dynamic light scattering (DLS) measurements were also conducted. As shown in Figure 2f, the average hydrodynamic radius of G-OF/PTP aggregates is 842.2 nm, which is much larger than that in the presence of CO_2 (104.1 nm). This is a direct indication that the G-OF/PTP aggregates were separated due to the electrostatic repulsion between G-OF and PTP. In order to further study the mechanism of our system, the fluorescence recovery of the hybrid probe G-OF/PTP with other gases in water was measured. As illustrated in Figure S4 in the Supporting Information, no fluorescence recovery was produced by N_2 or O_2 , indicating little interference from other gases in atmosphere. Moreover, the interference of SO_2 was checked as well. As illustrated in Figure S5, the emission of G-OF/PTP cannot be recovered in the presence of SO_2 , possibly resulting from the total quenching of G-OF by SO_2 which is different from that in the presence of CO_2 . Therefore, these results demonstrate a fluorescent, visible, and turn-on detection for CO_2 with high sensitivity and selectivity.

Taking the practicality into consideration, CO_2 is always present in the atmosphere and acts as an asphyxiant. Therefore, the fluorescence spectra of the G-OF/PTP hybrid probe before and after bubbling with CO_2/N_2 with different ratios were examined. As illustrated in Figure 3, the emission was recovered gradually with the increasing ratio of CO_2 in a series of CO_2/N_2 mixtures and the detected minimum ratio of CO_2 was 1.0% (Figure 3b). In practical terms, the maximal acceptable concentration was defined under 3.0%.³ Furthermore, the hybrid probe G-OF/PTP was also applied for solid-state

Figure 3. (a) Fluorescence spectra of G-OF in the presence of PTP before and after bubbling with CO_2/N_2 mixtures with different ratios for 2 min at a constant flow rate of 1.5 mL/min. (b) Plot of the fluorescence intensity of G-OF at 420 nm in the presence of PTP as a function of the ratio of CO_2 in CO_2/N_2 mixtures. $[\text{G-OF}] = 0.8 \mu\text{M}$, $[\text{PTP}] = 875.0 \text{ nM}$ (in repeat units), the volume ratios of CO_2/N_2 change from 0 to 100.0%. Error bars represent standard deviations of data from three separate measurements. Measurements were performed at 4 °C in water. The excitation wavelength was 378 nm.

sensing of CO₂. G-OF/PTP was sprayed onto a substrate of the cellulose ester film, and a dark blue to bright blue color change was observed under UV light upon exposure to CO₂ atmosphere (see Figure S6 in the Supporting Information). Therefore, our probe system offers a CO₂ detection approach allows for the assessment of the asphyxial risk.

Furthermore, the G-OF/PTP-based detection for CO₂ in gaseous phase was studied in confined spaces. As shown in Figure 4a, the detection solution of G-OF in water was placed

Figure 4. Scheme (a) and fluorescence spectra (b) of the G-OF with N₂ and CO₂ atmosphere in the presence of PTP. Scheme (c) and fluorescence intensities (d) of G-OF in CO₂ atmosphere in the presence of PTP versus different time of illumination. Blank represents the fluorescence intensities of G-OF in the presence of PTP in CO₂ atmosphere without photosynthesis. All data were presented as mean values $\pm$ standard deviation of three separate experiments. Error bars represent standard deviations of data from three separate measurements. [G-OF] = 0.8 μ M, [PTP] = 875.0 nM (in repeat units). Measurements were performed at 4 $^{\circ}$ C in water. The excitation wavelength was 378 nm.

in a confined chamber with CO₂ atmosphere (1.0 atm) for 12 h, and then the emission spectra of the G-OF/PTP hybrid probe was measured. Figure 4b exhibits that the gaseous CO₂ induces the significant recovery of emission through the absorption to the detection solution. Additionally, the different volume ratios of CO₂/N₂ mixtures in confined spaces were also detected, and the detected minimum ratio of CO₂ was 1.0% as well (see Figure S7 in the Supporting Information). In this way, the G-OF/PTP-based system allows for the detection of the gaseous CO₂ in confined spaces, which is extremely important for the workers in closed spaces to avoid the risk of asphyxia.

Finally, the CO₂ was monitored in the process of photosynthesis. It is well-known that plant photosynthesis converts CO₂ into carbohydrate by assimilation of sunlight and H₂O. Here, we employed *Zea mays*, a C₄-photosynthetic plant with high photosynthetic rate,^{51,52} as a model system to study the response of the G-OF/PTP-based probe to monitor the CO₂ changes in plant photosynthesis. As exhibited in Figure 4c, *Zea mays* was placed in the confined chamber with light and CO₂, and the fluorescence intensity of the G-OF/PTP hybrid probe was decreased gradually with the increase of the illumination time (Figure 4d), indicating the assimilation of CO₂ by photosynthesis. Therefore, our CO₂ detection strategy

can be applied to monitor CO₂ changes in plant photosynthesis.

CONCLUSIONS

In summary, we have designed a hybrid probe comprising guanidinium-pendent oligofluorene (G-OF) and water-soluble conjugated polythiophene derivative (PTP) for sensing CO₂ with very low background signal. This detection strategy takes advantage of the superquenching property of G-OF by PTP in the tight aggregates of G-OF/PTP, and the CO₂ controlled aggregation induces the turn-on signal of fluorescence. The new, simple, and light-up visible CO₂ assay system has several unique characteristics. First, the G-OF/PTP-based hybrid probe realizes the quantitative detection of CO₂ in both gaseous and dissolved phase. Second, the strategy can be applied for assessment of asphyxial risk in confined spaces. Furthermore, our CO₂ optical detection system enables the monitoring of CO₂ in plant photosynthesis. Therefore, the G-OF/PTP-based turn-on approach for CO₂ detection provides meaningful applications in asphyxia diagnosis and monitoring plant photosynthesis.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.6b01489.

Comparison of the strategies of “pre-mixing” (CO₂ was first mixed with G-OF) and “post-mixing” (CO₂ was first mixed with PTP); the fluorescence recovery of the hybrid probe G-OF/PTP with other gases; and detection of CO₂ by composite film and additional spectral figures (PDF)

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We are grateful for the financial supports from the National Natural Science Foundation of China (Grant No. 21574037), the “100 Talents” Program of Hebei Province, China (Grant No. E2014100004), the Program for Excellent Innovative Talents in Universities of Hebei Province, China (Grant No. BJ2014011), the Natural Science Foundation of Hebei Province (Grant No. B2015202330), the Tianjin Natural Science Foundation (Grant No. 15JCYBJC17500), and The Netherlands Organization for Scientific Research (NWO) with the Veni Grant (Grant No. 680-47-437). The authors acknowledge Prof. Shu Wang (Institute of Chemistry, Chinese Academy of Sciences) for valuable suggestions on this work.

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