

Highly Sensitive Fluorescent Probes for the Quantitative Determination of Singlet Oxygen ($^1\text{O}_2$)

Syed Rahin Ahmed, Kwangnak Koh, Nam Lyong Kang, and Jaebeom Lee*

Department of Nanofusion Technology, College of Nanoscience and Nanotechnology, Pusan National University, Miryang 627-706, Korea. *E-mail: jaebeom@pusan.ac.kr
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Singlet oxygen ($^1\text{O}_2$) is an important species for oxidation in biological processes. $^1\text{O}_2$ is implicated in the genotoxic effect, and plays an important role in the cell-signaling cascade and in the induction of gene expression. However, the rapid detection of $^1\text{O}_2$ in biological environments with sufficient specificity and sensitivity is hampered by its extremely low emission probability. Here, a layer-by-layer (LbL) film of CdTe quantum dots (QDs), polymers, and ascorbate have been designed as a rapid, highly selective, and sensitive fluorescence probe for $^1\text{O}_2$ detection. Upon reaction with $^1\text{O}_2$, the probe exhibits a strong photoluminescence (PL) response even at trace levels. This remarkable PL change should enable the probe to be used for $^1\text{O}_2$ detection in many chemical and biological systems and as an environmental sensor.

Key Words : Singlet oxygen, CdTe QDs, LbL method, Fluorescence, Quenching

Introduction

The chemistry of singlet oxygen ($^1\text{O}_2$) has received a lot of attention because of its unique role in many physiology and pathological processes. $^1\text{O}_2$ can react with many kinds of biological molecules such as DNA, proteins and lipids to cause degenerative diseases or to destroy malignant cells as a cancer therapy. It is now well established that singlet oxygen is an important oxidant in biological systems, and proteins are a major target for the damage initiated by this reactive species, which can exert a range of effects that can adversely affect cellular and tissue functions.

Recently, several research groups have offered improvement in the detection of singlet oxygen.¹⁻⁶ Khin K. Chin *et al.* reported the quantitative determination of $^1\text{O}_2$ upon excitation of tryptophan (Trp), tyrosine (Tyr), and phenylalanine (Phe), which is abundant light absorbers in the UV-B range (290-320 nm).⁷ Highly sensitive, selective and rapid fluorescent probe detection of $^1\text{O}_2$ has been developed by Kehua Xu *et al.*⁸ Over the last few years, the plasmon-induced enhancement of $^1\text{O}_2$ optical emission has been studied and is able to make significant changes in the emission properties of $^1\text{O}_2$.⁹⁻¹² Though a lot of effort has been made to detect $^1\text{O}_2$, an easier and cheaper detection method has so far been elusive. Layer by Layer (LbL) technology is advancing in such a way that they are becoming more portable, tunable, reliable and cheaper way for wide range of sensing applications.¹³ In this manuscript, we report on a highly sensitive fluorescence probe for the detection of ultra-low amounts of $^1\text{O}_2$ using the LbL technique.

Singlet oxygen is very electrophilic and reacts readily with reducing agents, such as ascorbate. Previous papers have shown that in neutral solutions singlet oxygen reacts with ascorbate (AscH-) to produce H_2O_2 ($1\ ^1\text{O}_2: 1\ \text{H}_2\text{O}_2$)¹⁴ and the fluorescence properties of CdTe quantum dots (QDs) can be

quenched by H_2O_2 because of the generation of surface defects which can prevent electron-hole recombination process.¹⁵ Careful observation of the fluorescence changes would help us in the quantitative determination of the $^1\text{O}_2$ produced in the system.

Materials and Methods

Poly(diallyldimethylammonium Chloride) (PDDA; M.W. 400,000-500,000), poly(acrylic acid) (PAA; M.W., ~450,000), Cadmium perchlorate hydrate, thioglycolic acid solution (TGA) and phenylalanine were obtained from Sigma-Aldrich. Aluminum telluride (Al_2Te_3) and ascorbic acid were acquired at the highest purity available from Cerac Company and Junsei Chemical Company respectively. All experiments were carried out in deionized (DI) water (>18 M).

SCINCO S-3100 series apparatus, using a cell with a 1-cm path length, was used to measure the UV-vis absorption spectra of the solutions. The photoluminescence spectra were recorded using a HITACHI F-7000 fluorescence spectrophotometer. The samples were excited at 380 nm, and the exciting slit and emission slit were 5 and 10 nm, respectively. The optical properties of the multilayer films were measured using a standard solid sample holder.

Preparation of CdTe QDs. CdTe QDs were synthesized as reported previously¹⁶. Briefly, $\text{Cd}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$ (0.985 g, 2.35 mmol) was dissolved in water (125 mL), and TGA (5.7 mmol) was added with stirring, followed by adjustment of the pH to the appropriate value (11.4-11.6) by dropwise addition of a 1 M solution of NaOH. The solution was placed in a three-necked flask and deaerated by N_2 bubbling for 30 min. Under stirring, H_2Te gas (generated by the reaction of Al_2Te_3 lumps (0.2 g, 0.46 mmol) with 0.5 M H_2SO_4 (15-20 mL) under a N_2 atmosphere) was passed through the solution together with a slow nitrogen flow for

20 min. The CdTe precursors formed were converted to CdTe nanocrystals by heating the reaction mixture under reflux at 100 °C for 20 min under open-air conditions with a condenser attached.

Preparation of LbL Films for Sensing. Typical microscopic glass slides (2×10 cm) were cleaned by immersion in piranha solution (3:1 H_2SO_4 : H_2O_2 , dangerous if in contact with organics) for 1 h, and then thoroughly rinsed with DI water and kept in DI water prior to use. Solutions of PDDA (1 wt %) and PAA (1 mg/mL) were prepared. The glass substrates were immersed consecutively in each of the PDDA and PAA solutions for 10 min. The washing process between consecutive adsorptions was carried out in copious amounts of DI water with N_2 gas drying to build the $(\text{PDDA}/\text{PAA})_3/\text{PDDA}$ of the base layers. Then, $(\text{CdTe QDs}/\text{PDDA})_{10}$ multilayers were prepared by alternate deposition of PDDA and CdTe QDs onto the substrates using an immersion time of 10 min followed by rinsing with water and drying under a N_2 flow after the deposition of each layer. For each cycle, a bilayer of PDDA/QDs was formed, and the UV-vis spectra of the growing layers were recorded in air after each assembly cycle. The substrates were immersed into $(\text{PDDA}/\text{PAA})_2/\text{PDDA}$ solutions to give spacer layer between the ascorbate and the fluorescent NPs to avoid any kind of interference. At the upper stage, two bilayers of PDDA/ascorbate were deposited. Finally, multilayers of CdTe QDs, polymers, and ascorbate (pH 13) were used for the sensing of $^1\text{O}_2$.

Results and Discussion

Singlet Oxygen Detection. Singlet oxygen was generated through direct photo-oxidation arising from the absorption of UV radiation by the chromophoric amino acid phenylalanine in an air-saturated D_2O solution. D_2O was used to detect the presence of $^1\text{O}_2$, because the lifetime of $^1\text{O}_2$ is ten times longer in D_2O than in H_2O . Samples were irradiated with a 266-nm pulse from a Nd:YAG laser. The initial species formed are the singlet states of Phe as a result of photo-ionization (eq. 1). These excited states are very short-lived, and readily lose energy via direct energy transfer to other groups by collisional deactivation and vibrations, and via intersystem crossing to the triplet state (eq. 2). The triplet state is much longer-lived than the singlet states, and is completed by energy transfer to oxygen in its triplet ground state (eq. 3). The sensitized singlet oxygen ($^1\text{O}_2$) generated reacts rapidly with ascorbate, producing H_2O_2 ¹⁴ (eq. 4). Finally, chemical etching of H_2O_2 on the QD layer will lead to surface defects; radiative recombination processes are avoided and fluorescence quenching will occur¹⁵ (eq. 5).

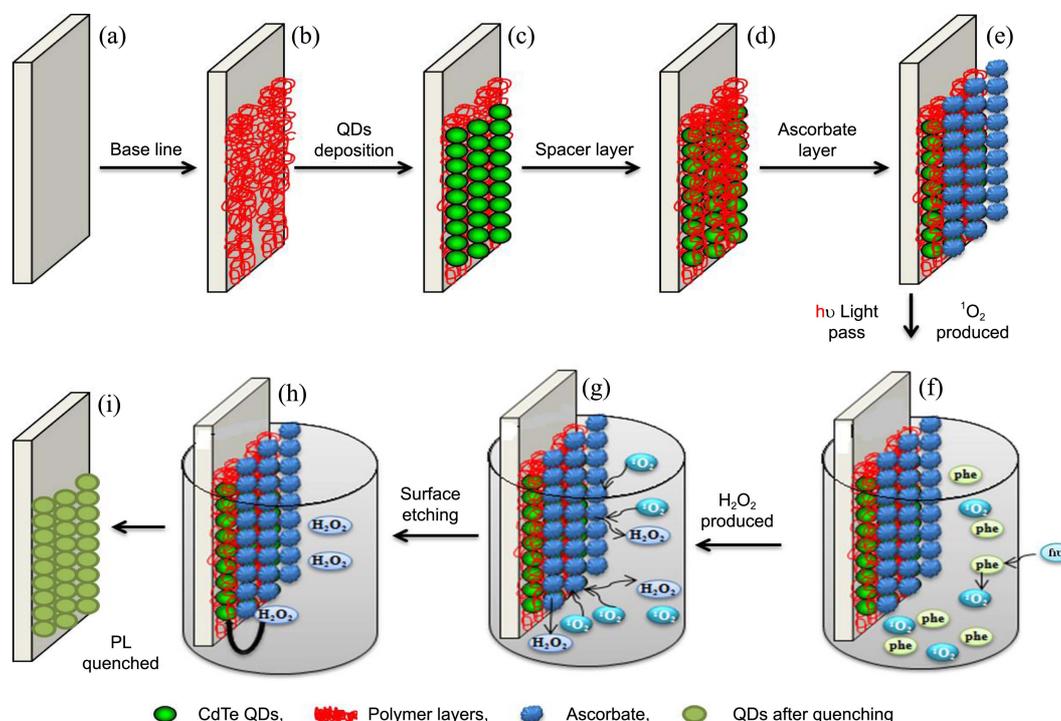
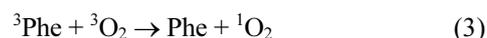


Figure 1. Schematic diagram of sensing assembly: (a) Cleaned glass slides to make LbL film; (b) Polymer layers deposited on glass slides to make base line; (c) QDs deposited on polymer layer; (d) Polymer layers deposited on QDs to give a space between fluorophore and ascorbate; (e) Deposited ascorbate at the top of the LbL film; (f) LbL film immersed in phenylalanine solution and UV light passed through the solution which produced $^1\text{O}_2$; (g) $^1\text{O}_2$ rapidly react with ascorbate and H_2O_2 produced; (h) H_2O_2 chemically etches the QDs and surface defects generated; (i) QDs quenched and lost its brightness.

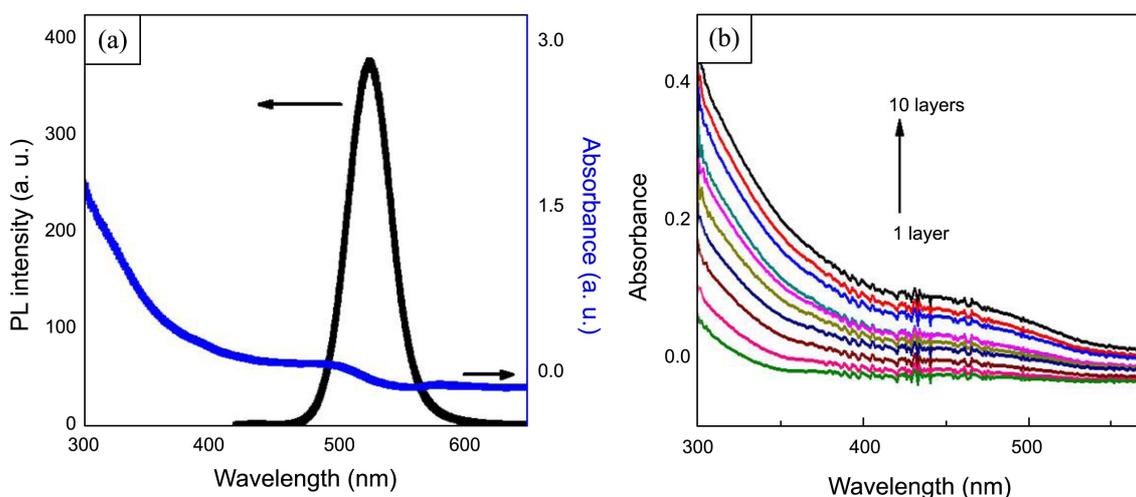


Figure 2. (a) UV-vis (blue line) & PL spectra (black line) of CdTe QDs; (b) UV-vis spectra of growing PDDA/CdTe QD multilayers.

The schematic presentation of overall sensing process is shown in Figure 1.

Spectroscopic Studies of CdTe QDs and (PDDA/QDs) Multilayer Films. Figure 2(a) shows typical absorption and photoluminescence (PL) spectra of the synthesized CdTe QDs. The absorbance shoulder of the QDs is located at 510 nm, while the photoluminescence peak of the QDs is situated at 534 nm. The LbL assembly process of the PDDA and QDs was monitored by the absorbance increase in the UV-vis absorption spectra. Figure 2 shows the UV-vis spectra of the (PDDA/CdTe QD)₁₀ multilayers deposited on a glass

substrate, where the first layer is PDDA and the outermost layer contains the QDs. The absorbance shoulder at 510 nm increases steadily with the number of bilayers, confirming the irreversible adsorption of the PDDA and QDs on the substrate. The absorption at 510 nm for LbL film originated from QDs tells about the approximate sizes of the nanocrystals.

Spectroscopic and Microscopic Studies Nanocomposite Films for ¹O₂ Sensing. The sensing assembly of ¹O₂ was made as shown in Figure 1. Figure 3(a) shows the spectra of the LbL films before and after incubation with phenyl-

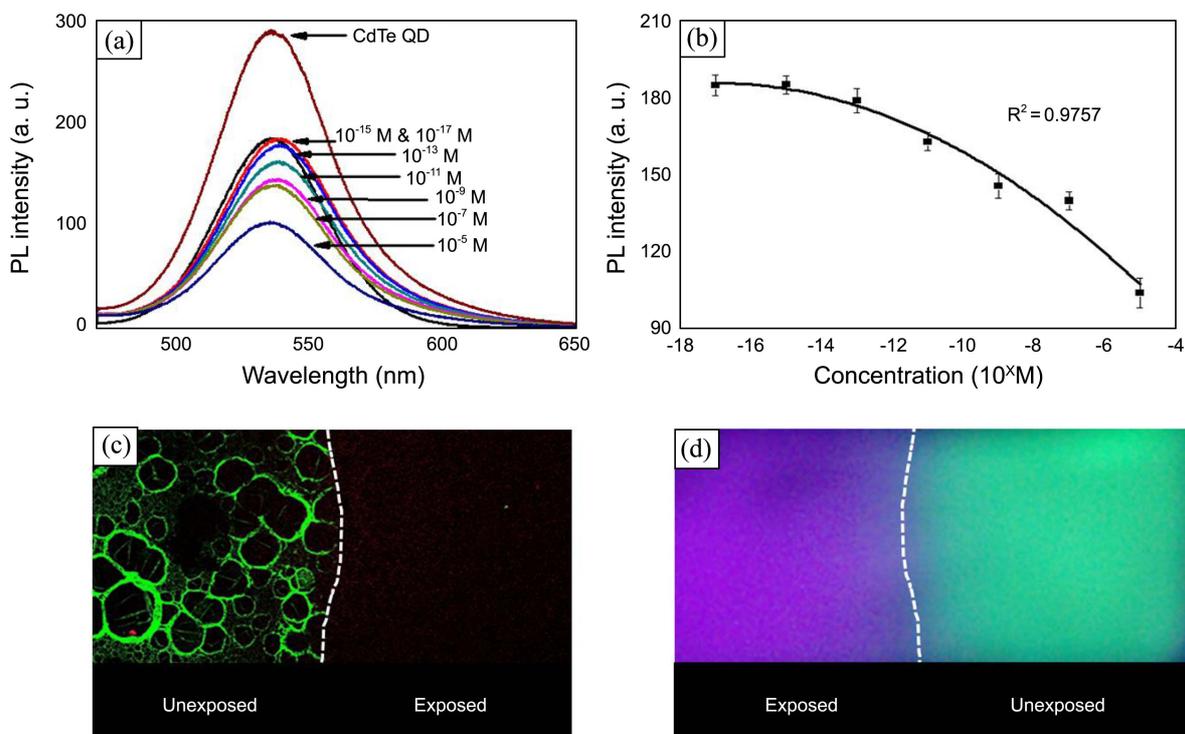


Figure 3. (a) Concentration dependant PL changes of LbL film (b) A plot of PL changes with concentration (Polynomial fitting function is utilized to find out regression factor) (c) Microscopic image of interface between exposed (right side) and unexposed (left side) part (d) UV photograph of exposed (left side) and unexposed (right side) part at the concentration of 10^{-15} M.

alanine. Before incubation, the multilayer has a strong photoluminescence peak at 534 nm. After incubation, H_2O_2 is produced into the solution (eq. (1)-(4), above) and a significant decrease in the photoluminescence peak at 534 nm is observed instantly (as shown in Figure 3(a)). Such photoluminescence quenching originates from the production of surface defects on the QDs by H_2O_2 , which can prevent radiative recombination of electrons and holes by trapping these carriers. Moreover, the quenching of the photoluminescence intensity of the multilayers is dependent on the H_2O_2 concentration. The photoluminescence intensity is quenched with decreasing H_2O_2 concentration, and was saturated at 10^{-15} M (as shown in Figure 3(b)). The quenching of the LbL films can be visualized easily with the naked eye. A fluorescence microscope (Leica DM 2000) was used to acquire the fluorescence micrographs. Fluorescence microscopic images at the interface between the exposed (right side) and unexposed (left side) parts are shown in Figure 3(c). Figure 3(d) shows UV photographs of the films after H_2O_2 exposure. After half of the coated glass (upper part) was incubated with solution for 1 min, the photo-

luminescence of the part exposed to H_2O_2 was totally quenched, while the photoluminescence of the unexposed part (lower part) remained unchanged. This property allows the direct determination of the concentration of $^1\text{O}_2$, which can produce H_2O_2 (1 $^1\text{O}_2$:1 H_2O_2), through measurement of the rate of decrease of the photoluminescence intensity.

Figure 4(a) shows the time-dependent changes in fluorescence intensity for the LbL films upon interaction with 1×10^{-15} M H_2O_2 . The photoluminescence intensity of the films changed for times up to 5 min, after which it reached a steady position. This might be due to the reactivity of H_2O_2 against the QDs. Therefore, we used 5 min as the typical response time in our study in order to observe the quenching rate. The absolute quenching rate ($F_0 - F_m$) was taken at 534 nm between the initial fluorescence intensity (F_0) and the fluorescence intensity (F_m) 5 min after the surface etching, and then the absolute quenching rate *per minute* of the sensing assembly, Q_m , was calculated for the first 5 min using the following equation:

$$Q_m = \frac{(F_0 - F_m)}{5}$$

The quenching rate of the photoluminescence intensity for the first 5 min against the H_2O_2 concentration is plotted in Figure 4(b). The absolute quenching rate decreases linearly with decreasing H_2O_2 concentration up to a concentration of 1×10^{-15} M. When the concentration of H_2O_2 is below this value, the curve no longer decreases and appears saturated.

Conclusion

In summary, we have developed a fluorescent probe for a monitoring system for $^1\text{O}_2$ based on the LbL technique. The photoluminescence properties of CdTe QDs and their sensitivity toward $^1\text{O}_2$ were tested. The results suggested that the response of the probe to $^1\text{O}_2$ was rapid and highly sensitive. Fluorescence microscopy experiments showed that the probe could be effective for detecting the presence of $^1\text{O}_2$. Therefore, the probe proposed here could be broadly applicable to the quantitative determination of $^1\text{O}_2$ in many chemical and biological systems.

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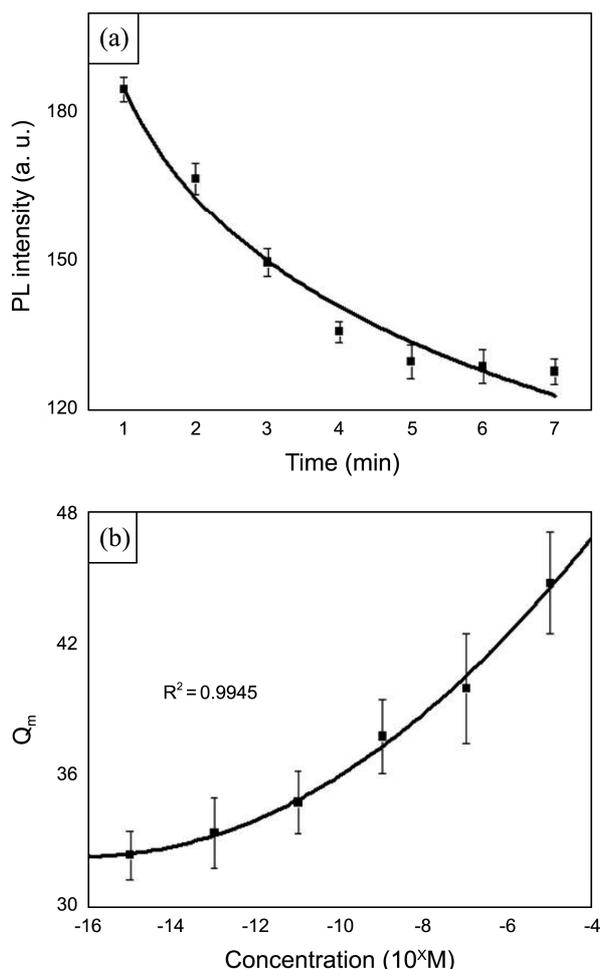


Figure 4. (a) Time dependant fluorescence change of LbL films upon interaction with 1×10^{-15} M H_2O_2 ; (b) Absolute quenching rate of PL intensity within 5 min as a function of H_2O_2 concentration (Polynomial fitting function is utilized to find out regression factor).

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