Caging and photo-triggered uncaging of singlet oxygen by excited state engineering of electron donor–acceptor-linked molecular sensors

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Singlet oxygen (1O2), one of the most sought-after species in oxidative chemical reactions and photodynamic cancer therapy, is activated and neutralized in the atmosphere and living cells. It is essential to see “when” and “where” 1O2 is produced and delivered to understand and utilize it. There is an increasing demand for molecular sensor tools to capture, store, and supply 1O2, controlled by light and engineered singlet and triplet states, indicating the 1O2-capturing-releasing state. Here, we demonstrate the outstanding potential of an aminocoumarin-methylanthracene-based electron donor–acceptor molecule (1). Spectroscopic measurements confirm the formation of an endoperoxide (1-O2) which is not strongly fluorescent and remarkably different from previously reported 1O2 sensor molecules. Moreover, the photoexcitation on the dye in 1-O2 triggers fluorescence enhancement by the oxidative rearrangement and a competing 1O2 release. The unique ability of 1 will pave the way for the spatially and temporally controlled utilization of 1O2 in various areas such as chemical reactions and phototherapies.

Singlet oxygen (1Δg) (1O2), the lowest excited state of molecular oxygen, is an essential member of the reactive oxygen species (ROS) family and an active intermediate in various chemical and biological reactions1–5. Uncontrolled production of 1O2 causes undesirable degradation of materials and oxidative stress-induced disease progression. Therefore, the controlled and localized generation and sensing of 1O2 are essential and beneficial for utilizing 1O2 in chemical and biological reactions arbitrary.

1O2 sensing is important to detect and control its reactions, such as in PDT to kill cancer cells or in fine chemicals synthesis6–10. Fluorogenic sensing is one of the most efficient methods for 1O2 detection because of its high sensitivity5,7. One of the most promising fluorescence sensors of 1O2 is based on a fluorophore-spacer-1O2 receptor system. Anthracene moiety is often chosen as an excellent receptor owing to its high selectivity and reactivity toward 1O25. Such sensors are nonfluorescent before reacting with 1O2 due to efficient photoinduced intramolecular electron transfer (PET). Cycloaddition between 1O2 and a sensor affords an endoperoxide, blocking the PET and uncaging the emission5.

Apart from the 1O2 sensing, the demand for capturing and controlled releasing of 1O2 has also been studied significantly in various areas in biology1–3 and chemistry4. However, it is challenging to produce it in the hypoxic tumor microenvironment1–3. This challenge is examined by stimuli-induced releasing of 1O25,16. Conventionally, 1O2 was liberated by heating endoperoxides of acenes or piperidones10–14. Fudickar et al. developed a dipyridylanthracene endoperoxide and released 1O2 under a chemical trigger13. Ucar et al. demonstrated a two-step chemical stimulus-induced release of 1O2 from naphthalene endoperoxide14. Phototriggered 1O2 release is also reported by the photoexcitation on the anthracenyl part of the endoperoxide, although the high energy light of 282 nm laser was used16. Despite many 1O2 sensor molecules reported17–21, the sensor shows unexpected capture,
store, and supply \( ^1\text{O}_2 \), indicating the capturing-releasing states with > 50-fold fluorescence intensity enhancement from the D-A form to the caged form and the endoperoxide.

Herein, the present study demonstrates the molecular dyad system that chemically traps, optically releases, and efficiently senses \( ^1\text{O}_2 \) in a temporally controlled manner. It is identified that an aminocoumarin-methylanthracene-linked molecule (1) traps \( ^1\text{O}_2 \), to form the endoperoxide (1-\( ^1\text{O}_2 \)). Remarkably, 1-\( ^1\text{O}_2 \) is not as fluorescent as commercially available fluorogenic \( ^1\text{O}_2 \) probe molecules with an anthracenyl moiety. It is indicated in the present study that the unique molecular orbitals and triplet excitation energy levels of 1-\( ^1\text{O}_2 \), making the efficient \( ^1\text{O}_2 \) capturing, storing, and releasing with fluorescence sensing achievable by one- or two-photon excitation. These unique phenomena are verified using spectroscopic measurements, including NMR and EPR, and density functional theory (DFT) calculations.

**Methods**

**General.** All chemicals used in this research were analytical grade and used as received unless otherwise stated. Potassium carbonate \((\text{K}_2\text{CO}_3)\), potassium iodide \((\text{KI})\), hydrochloric acid \((\text{HCl})\), and sodium azide \((\text{NaN}_3)\) were obtained from FUJIFILM Wako Pure Chemical Corporation, Japan. 7-Amino-4-methyl coumarin, 7-ethylamino-4-methyl coumarin, 9-chloromethyl anthracene, 9-methanthracene, tetrakis(4-carboxyphenyl)porphyrin \((\text{TCPP})\), and Rose Bengal \((\text{RB})\) from Tokyo Chemical Industry \((\text{TCI})\), Japan. We obtained 2,2,6,6-tetramethylpiperidine-1-oxyl \((\text{TEMPO})\) from Sigma Aldrich, USA. SOSG was obtained from Sigma and SiDMA sensor from DOJINDO, Japan. All the solvents were in reagent grade and from FUJIFILM Wako Pure Chemical Corporation, Japan.

Absorption spectra were recorded using an Evolution 220 UV–visible spectrophotometer \((\text{ThermoFisher Scientific})\), and fluorescence (FL) spectra were recorded using a Hitachi F-4500 FL spectrophotometer. NMR measurements were performed using an Agilent Unity INOVA 500 or JEOL ECX-400 spectrometers. The continuous-wave-EPR measurements were carried out using a Bruker EMXplus spectrometer. For photoirradiation of samples, we used a DPSS 532 nm Green laser \((\text{Shanghai Dream Laser Technology})\), a Xenon/ Mercury lamp \((\text{Hamamatsu Photonics KK, Japan})\), or an 800 nm femtosecond laser \((\text{Coherent Mira 900, the pulse width is 40 fs})\).

**Synthesis and characterization.** 1 was prepared and characterized according to the reported procedure with slight modification\(^{20}\). 7-Amino-4-methylcoumarin \((0.175 \text{ g}, 1.00 \text{ mmol})\) and 9-chloromethylanthracene \((0.227 \text{ g}, 1.00 \text{ mmol})\) were dissolved in 20 mL of acetonitrile. Then, \(\text{DBU} \) \((304 \text{ mg}, 2.00 \text{ mmol})\) was added to the solution, and the reaction mixture was stirred at 82 °C for 6 h. The reaction mixture was cooled to room temperature, and excess water was added, which provided a yellow residue. The pH of the solution was adjusted to ~6–7 using \(\text{aq. HCl} \). The residue was filtered and dried. The yellow powder was re-dissolved in hot \(\text{THF} \) and then reprecipitated by adding an excess of toluene, and the residue was filtered and washed with toluene and then with acetone to give a pale-yellow powder \((0.332 \text{ g}, 92\%)\). \(\lambda_{\text{max}} \) \((\text{DMF})\): 354, 370, 389 nm. 1: \(\text{H NMR} \) \((400 \text{ MHz, CDCl}_3)\): \(\delta = 8.50 \ (1 \text{ H}), 8.21 \ (2 \text{ H}), 8.05 \ (2 \text{ H}), 7.57–7.40 \ (m, 5 \text{ H}), 6.74 \ (1 \text{ H}), 6.58 \ (1 \text{ H}), 6.02 \ (s, 1 \text{ H}), 5.21 \ (2 \text{ H}), 4.30 \ (1 \text{ H}), 2.40 \ (3 \text{ H})\).

2 was prepared according to the process as follows. 7-(Ethylamino)-4-methylcoumarin \((0.10 \text{ g}, 0.49 \text{ mmol})\) and 9-chloromethylanthracene \((0.16 \text{ g}, 0.73 \text{ mmol})\) were dissolved in 10 mL of \(\text{DMF} \). Then, \(\text{K}_2\text{CO}_3 \) \((47 \text{ mg}, 0.29 \text{ mmol})\) and potassium iodide \((5 \text{ mg}, 0.03 \text{ mmol})\) were added to the solution, and the reaction mixture was stirred at 85 °C for 5 h. The reaction mixture was cooled to room temperature, and excess water was added, which provided a yellow residue. The pH of the solution was adjusted to ~6–7 using \(\text{aq. HCl} \). The residue was filtered and dried. The yellow powder was re-dissolved in hot \(\text{THF} \) and then reprecipitated by adding an excess of toluene, and the residue was filtered and washed with toluene and then with acetone to give a pale-yellow powder \((0.332 \text{ g}, 92\%)\), \(\lambda_{\text{max}} \) \((\text{DMF})\): 354, 370, 389 nm. 1: \(\text{H NMR} \) \((500 \text{ MHz, CDCl}_3)\): \(\delta = 8.52 \ (s, 1 \text{ H}), 8.14–8.16 \ (d, 2 \text{ H}), 8.05–8.07 \ (d, 2 \text{ H}), 7.55–7.48 \ (m, 5 \text{ H}), 6.95–6.98 \ (d d, 1 \text{ H}), 6.91–6.92 \ (d, 1 \text{ H}), 6.05 \ (s, 1 \text{ H}), 4.30 \ (s, 2 \text{ H}), 3.06–3.10 \ (q, 2 \text{ H}), 2.42 \ (s, 3 \text{ H}), 0.77–0.80 \ (t, 3 \text{ H}), 3.93 \ (3 \text{ H})\).

**Steady-state FL and absorption spectroscopic studies of \( ^1\text{O}_2 \) sensing.** A sample solution of a sensor molecule \((1 \text{ or } 2; 10.0 \mu\text{M})\) and a photosensitizer \((5.00 \mu\text{M})\) in \(\text{DMF} \) was photoexcited under selective photosensitizer excitation. The sample solution containing \(\text{RB} \) was irradiated with a 532 nm \(\text{DPSS, 50 mW} \) continuous-wave laser. That containing TCPP was illuminated with a xenon lamp fitted with a 410–430 nm bandpass filter or a 404 nm \(\text{Thorlabs, 70 mW} \) continuous wave laser. The sample solution was irradiated with a UV LED lamp \((\text{Asahi-spectra. Co. CL})\) \((365 \text{ nm}, 10 \text{ nm band path, 1.0 mW cm}^{-2}\)). The FL and absorption spectra were recorded before and after irradiation.

**Isolation and characterization of the product of the reaction of 1 and \( ^1\text{O}_2 \).** 2.0 mM of 1 and 1.0 mM of \(\text{RB} \) were mixed in 800 μL of \(\text{DMF} \) (HPLC grade) and illuminated by a green diode laser \((532 \text{ nm, 50 mW}, 10 \text{ min})\). The reaction mixture was subjected to an HPLC system \((\text{Agilent 1220})\) equipped with a C18-MS-II column \((\text{Nacalai}; 4.6 \text{ mm I.D. × 250 mm})\) using \(\text{DMF} \) as the eluent. The fraction with the peak retention time of 2.8 min was collected and removed the solvent in a vacuum in the dark. DMSO-\(d_6 \) was added and measured by an NMR spectrometer. Yield 86% \((\text{estimated from the HPLC profile})\), \(\lambda_{\text{max}} \) \((\text{DMF})\): 354 nm, \(\text{H NMR} \) \((400 \text{ MHz, CDCl}_3)\): \(\delta = 8.50 \ (1 \text{ H}), 8.21 \ (2 \text{ H}), 8.05 \ (2 \text{ H}), 7.57–7.40 \ (m, 5 \text{ H}), 6.74 \ (1 \text{ H}), 6.58 \ (1 \text{ H}), 6.02 \ (s, 1 \text{ H}), 5.21 \ (2 \text{ H}), 4.30 \ (1 \text{ H}), 2.40 \ (3 \text{ H})\).
DMSO-d$_6$ δ = 7.53–7.56 (m, 4 H), 7.49 (s, 1H), 7.31–7.33 (m, 4H), 6.98 (d, J = 9.1 Hz, 1H), 6.92 (s, 1H), 6.86 (t, J = 4.1 Hz, 1H), 6.47 (s, 1H), 5.98 (s, 1H), 4.50 (d, J = 4.1 Hz, 2H), 2.35 (s, 2H).

The reaction mixture without the HPLC separation were also measured for comparison. And then, the sample solutions were irradiated with a UV LED lamp (Asahi-spectra. Co. CL) (365 nm, 10 nm band path, 1.0 mW cm$^{-2}$, 10 min) and again measured by the NMR spectrometer. The results were shown in Figs S6 and S7.

Estimation of the singlet oxygen photo-releasing quantum yield from the EPR studies. The singlet oxygen photo-releasing quantum yield is estimated to be 1.6% based on the absorbed number of photons (320 nmol) and the detected 1O$_2$ (10.0 nmol × 50% = 5.00 nmol). The absorbed number of photons was calculated based on the induced light and absorbancy of the sample solution after the photosensitization of RB. The detected 1O$_2$ in mole was calculated based on the used 1 (10 μM, 0.50 mL) and the signal change ratio of TEMP to be TEMPO (50%).

Steady-state FL and absorption spectroscopic studies for 1O$_2$ releasing. A sample solution of 1 (10.0 μM) and a photosensitizer (5.00 μM) in DMF was photosensitized under selective photosensitizer excitation. The sample solution containing RB was irradiated with a 532 nm (50 mW) continuous-wave laser. Then, SOSG (10 μM) was added and the sample solution was irradiated with a UV LED lamp (Asahi-spectra. Co. CL) (365 nm, 10 nm band path, 1 mW cm$^{-2}$). Before adding SOSG, the solution was purged with argon (50 mL/min, 20 min) for the condition of the absence of oxygen. The FL and absorption spectra were recorded before and after irradiation.

Steady-state FL and absorption spectroscopic studies under NIR-activation of the intermediate complex. A sample solution containing 1 (10.0 μM) and rose bengal (10.0 μM) in DMF was irradiated under a 532 nm green laser (50 mW) for the photosensitized generation of 1O$_2$. The sample’s FL and absorption spectra (250 μL in 5 mm pathlength cuvette) were recorded before and after 30 min of the photosensitization. Then, the sample solution was irradiated with an 800 nm fs laser (Coherent Mira 900) for 40 min, and FL and absorption spectra were recorded every 5 min interval. The samples’ FL quantum yield was estimated by a relative FL quantum yield estimation using coumarin 120 as a reference. A control experiment was conducted to compare the enhancement factor by recording the FL spectra of the equivalent sample solution after photosensitization, which was kept under dark.

Steady-state FL and absorption spectroscopic studies for 1O$_2$ sensing. A sample solution of a sensor molecule (1 or 2: 10.0 μM) and a photosensitizer (5.00 μM) in DMF was photosensitized under selective photosensitizer excitation. The sample solution containing RB was irradiated with a 532 nm (DPSS, 50 mW) continuous-wave laser. That containing TCPP was illuminated with a xenon lamp fitted with a 410–430 nm bandpass filter or a 404 nm (Thorlabs, 70 mW) continuous wave laser. The sample solution containing RB was irradiated with a UV LED lamp (Asahi-spectra. Co. CL) (365 nm, 10 nm band path, 1.0 mW cm$^{-2}$). The FL and absorption spectra were recorded before and after irradiation.

Density functional theory (DFT) calculations. The molecular structures and electron energies were optimized and obtained by the Gaussian16 package$^{22}$ using ub3lyp/6-311 + + G** level of theory$^{23,24}$. Molecular orbital analyses were performed for the natural transition orbitals$^{25}$ using “Pop = (NTO,SaveNTO)” and “Density = (Check,Transition = n)” keywords after performing TD-DFT calculations.

Electron paramagnetic resonance (EPR) studies. The generation of 1O$_2$ was indirectly monitored by using a spin probe TEMP that undergoes oxidation by 1O$_2$ to form EPR-active TEMPO. The measurement conditions were optimized by evaluating the photosensitization of RB in the presence of TEMP. To this purpose, 5 mM of TEMP was added to 5.00 μM RB in DMF. The solution was irradiated with a xenon lamp fitted with > 480 nm long-pass filter for 30 min (50 mW at 532 nm). After the photosensitization, the EPR spectra of the sample solution were recorded using the X band frequency of microwave (9.79 GHz) at 1 mW cm$^{-2}$ power. To check the possibility of generation 1O$_2$ under UV illumination, 1 or RB was illuminated with a UV lamp with an emission maximum at 365 nm, at 2.0 mW cm$^{-2}$ for 10 min in the presence of 5.00 mM of TEMP.

To examine the UV-activated release of 1O$_2$, a sample solution containing 1 (10 μM) and RB (5 μM) was irradiated with a xenon lamp fitted with > 480 nm long-pass filter for 30 min (50 mW at 532 nm). After the photosensitization and generation of the intermediate complex, 5 mM of TEMP was added to the sample solution, and EPR spectra were recorded before and after 10 min of UV illumination (365 nm, 10 nm band path, 2 mW cm$^{-2}$). A control experiment was conducted by illuminating a sample solution containing 1 (10 μM) and RB (5 μM) and 5 mM of TEMP with UV light (UV, 2 mW cm$^{-2}$ at 365 nm).

The enhancement factor of the EPR signals was determined by assuming the formation of TEMPO in the presence of TEMP and RB without 1 to be 100% (Fig. S7).

Results and discussion

Reactions of compound 1 with 1O$_2$ generated by a photosensitizer. The anthracene-based electron donor–acceptor molecule possessing a coumarin chromophore (1) (Fig. 1a) was synthesized by a one-step reaction started from 7-amino-4-methylcoumarin and 9-chloromethylanthracene, and characterized by the spectroscopic methods including NMR spectrometry (see “Methods” section). First, we confirmed that 1 is nonfluorescent because of the intramolecular electron transfer from the anthracene moiety to coumarin moi-
1 reacts with $^{1}\text{O}_2$ generated by the photosensitization of Rose Bengal (RB) by green light to form a moderately fluorescent endoperoxide 1-$\text{O}_2$ (Fig. 1). The formation of 1-$\text{O}_2$ was confirmed by 1D and 2D NMR measurements on the isolated product of the reaction. The NMR spectra help us to confirm the formation of 1-$\text{O}_2$. The high-field shift of the signals corresponding to the anthracenyl moiety indicated the breaking of the large pi-conjugation due to the endoperoxide formation, without shifting the signals to the coumarin moiety (Figs. S6 and S7). The observed correlation further supports this assignment among the signals in the NOESY spectra (Fig. S7). One of the characteristic correlations is observed between 4.50 ppm and 6.86 ppm from the methylene protons and the proton attached to the nitrogen atom. Also, a correlation between 4.50 ppm and 7.53–7.56 ppm from the methylene protons and the protons on the anthracenyl moiety evidences the justification of the attribution shown in Fig. S6.

The negative values of the calculated relative free energies of formation at the UB3LYP/6-311 + + G** level on 1-$\text{O}_2$ (−0.66 kcal/mol relative to 1 and $^{1}\text{O}_2$) also guarantee the feasibility of the formation of 1-$\text{O}_2$ (Fig. S8). Absorption spectroscopic observations provide information on the reaction products. Figure 1b shows the absorption spectra of 1 recorded as a function of time under the photosensitization of RB. The intensities of the anthracene's vibrational bands at 390 nm and 370 nm are decreased by the 1$\text{O}_2$ mediated oxidation of the anthracenyl moiety. After the UV illumination, the absorbance around 290 nm was remarkably increased, suggesting the formation of the endoperoxide 1-$\text{O}_2$.

Remarkably, the fluorescence quantum yield of 1-$\text{O}_2$ was found to be $\phi \sim 0.03$ and is not as high as commercially available fluorogenic $^{1}\text{O}_2$ probe molecules which form the endoperoxide in the fluorescent form ($\phi > 0.5$) although the original dye part of 1-$\text{O}_2$ (i.e., 7-amino-4-methyl coumarin; Coumarin 120) is highly fluorescent ($\phi = 0.62$). This result implies the huge fluorescence intensity enhancement possibility during $^{1}\text{O}_2$ sensing and release of $^{1}\text{O}_2$.
the existence of a non-radiative relaxation pathway in the photoexcitation of 1-O₂, which is discussed below. Interestingly, a short-time illumination of UV light (365 nm, 1.0 mW cm⁻²) on both the reaction crude between 1 and 1-O₂ or the isolated 1-O₂, induced the remarkable fluorescence intensity enhancement (Fig. 1c and d). A 45-fold increase in the emission intensity from the starting 1 occurred by 3 min UV light illumination. This change inspired us to understand the formation of the products in the reaction of 1 and 1-O₂.

The changes in the absorption and emission characteristics between 1-O₂ and the final product(s) suggest that the UV illumination significantly accelerated the change in the molecular structure. Anthraquinone is one of the resultant products after UV illumination due to an oxidative rearrangement, supported by the fact that the UV illumination significantly accelerated the change in the molecular structure. Anthraquinone is not involved in the frontier orbitals, and it does not participate in the UV-light triggered emission intensity enhancement. Furthermore, to investigate the contribution of the anthracene moiety for the emission intensity enhancement, we examined the photoresponse to 1 under the UV illumination in the presence or absence of RB (Fig. S9). Relative to the UV illumination on 1-O₂, little change was observed in either case, ruling out the significant contribution of the photosensitization. Thus, the UV light-induced intensity enhancement is due to an intramolecular process, which would be the formation of the fluorogenic product rather than photodimerization.

Notably, 1-O₂ is stable in the dark room temperature conditions for more than 24 h. 1-O₂ was found to be stable, which is evident from the UV-induced enhancement of the emission intensities of the sample stored in the dark for 24 h or longer (Fig. 1c and d). We examined its thermal stability by heating it at 100 °C for 30 min under air. The emission spectra remained unchanged after the heating, indicating the high thermal stability of 1-O₂ (Fig. S10).

DFT calculations correlated with the fluorescence uncaging of 1-O₂. Next, the reasons for the weak fluorescence of 1-O₂ were studied through the DFT calculations. The localization of HOMO and LUMO on the coumarin moiety of 1-O₂ supports the most feasible transition in these orbitals through the photoexcitation (Fig. 3a). It is also indicative of the intramolecular electron transfer. The localization of HOMO and LUMO on the coumarin moiety is not involved in the frontier orbitals, and its energy is comparable to the S₁ state (Fig. 3b). It is also photoreactive with the 532 nm laser and the UV illumination. The emission intensity remained unchanged during the following UV illumination (Fig. 2b). A similar result was observed in the experiment using the sample solution purged with argon prior to the illumination (Fig. S3). This result verifies the essential participation of 1O₂ to form 1-O₂.

Meanwhile, 1-O₂ showed substantial stability towards the 1O₂ scavenger Na₂S (Figs. 2a-d and S5). Figure 2a shows the emission spectra of the sample solution containing 1 and RB with 15 eq. of Na₂S. The spectra represent the changes during the photosensitization with the 532 nm laser and the UV illumination. The emission intensity remained almost unchanged during the following UV illumination (Fig. 2b). A similar result was observed in the experiment using the sample solution purged with argon prior to the illumination (Fig. S3). This result verifies the essential participation of 1O₂ to form 1-O₂.
framework would play a crucial role in achieving the UV-induced emission intensity enhancement. Furthermore, the DFT calculations conclude that the substituted anthracenyl moiety contributes to the unique low fluorescence of $1-O_2$, which is definitively different from the other reported donor–acceptor type $1O_2$ sensor molecules reported so far. Therefore, it is an excellent example showing the fluorescence on/off switching property achieved by the excited-state engineering of molecules.

**The UV-induced uncaging of $1O_2$ in 1.** EPR is one of the most widely employed techniques to study the reactions involving $1O_2^{35,36}$. Remarkably, the UV (365 nm) illumination triggers $1O_2$ release in parallel with the fluorescence intensity enhancement, as evidenced by the EPR results (Fig. 4) correlated with the fluorescence and absorption results. A solution containing 1 and RB was illuminated in the presence of a spin probe TEMP, which has the sterically hindered amine to monitor $1O_2$ since the oxidation of the probe generates an EPR-active N-oxyl radical, TEMPO$^{36}$. The EPR spectra were recorded before and after UV illumination following the photosensitization by RB. A remarkable enhancement of the EPR signal intensity, which indicates the formation of TEMPO, was observed after the UV illumination (Fig. 4a and b). The control experiments without the photosensitization of RB did not give any EPR signal enhancement triggered by the UV illumination (Fig. 4c and d).

**Figure 2.** Temporal controlled detection of $1O_2$ by 1. (a) Fluorescence spectra ($\lambda_{ex}: 340$ nm) of a solution of 1 ($10 \mu$M) and RB ($5 \mu$M) before and after every 5 min of photosensitization (352 nm, 50 mW) and photoactivation with the UV light (365 nm, 1 mW cm$^{-2}$) in DMF, and in the presence of $1O_2$ scavenger NaN$_3$. (b) Time-traced relative emission intensities at 420 nm. The red and blue bars indicate the illumination time points by 532 and 365 nm lights, respectively. (c) Fluorescence spectra of a solution of 1 and RB in DMF before and after the photosensitization (352 nm, 50 mW) for 30 min, followed by the addition of NaN$_3$ (150 µM) and photoactivation with the UV light (365 nm, 1 mW cm$^{-2}$). (d) Time-traced relative emission intensities at 420 nm of the sample solutions containing NaN$_3$ (black) and without NaN$_3$ as a control experiment (red). (e,f) The UV-induced changes to the emission intensity after storing $1O_2$ at different times ranging from 30 min to 24 h. The arrows indicate the starting time point of the UV-light excitation for each condition.
We also verify that neither 1 nor RB generates 1O2 under the UV illumination (Fig. S14), and TEMP itself does not produce or release 1O2 (Fig. S15). Moreover, the same phenomena, the 1O2 detection only after the photosensitization of RB in the presence of 1, were also observed by using SOSG or SiDMA (Figs. S16 and S17). Because the 1O2 detection by SOSG was also observed with removing O2 after the photosensitization, the uncaging of 1O2 from 1-O2 was indicated (Fig. S17b). Thus, it was concluded that the phototriggering releases the caged 1O2 in 1-O2, along with the formation of fluorescent products (Figs. 1a and S6). Also, we used the commercial sensor SiDMA for examining the 1O2 release. The absorbency of SiDMA is decreased and disappeared (Fig. S18).

It should be noted that the applied wavelength of light is significantly longer (hv: 365 nm), at which anthracene endoperoxide does not possess optical absorption, than those in the previous reports showing the phototriggered release of 1O2 by the illumination (hv: 282 nm)16 on the anthracene moiety16,26. Furthermore, the release was also detected by two-photon excitation. The linked dye molecule of 1, coumarin, would change the usable wavelength of light for the phototriggered release from the endoperoxide. The enhancement factor of the EPR signal is ca. 50% relative to the control using TEMP and RB without 1 (Fig. S14), which suggests ca. 50% 1-O2 photoreleased 1O2. The remaining 50% 1-O2 would convert to the fluorescent coumarin derivative as mentioned above with the 1H-NMR measurements or be retrapped by unreacted 1 in the sample solution. The singlet oxygen photo-releasing quantum yield is estimated to be 1.6% based on the absorbed number of photons (320 nmol) and the detected 1O2 (10.0 nmol × 50% = 5.00 nmol).

NIR two-photon induced reaction of 1 with 1O2. The NIR-active molecular systems promise phototherapy, photo-uncaging-mediated drug delivery, and efficient chemical reactions because of the permeability of cells and tissues to NIR light37–40. In this context, we investigated the release of 1O2 from 1-O2 under a pumped-pulsed NIR laser activation since coumarins possess a moderate two-photon absorption cross-section41. First, we verified that one-photon excitation under 404 nm laser excitation activated 1-O2 (Fig. S19). Then, we examined the fluorescence and molecular structural features of 1-O2 during two-photon excitation with an 800 nm pulsed laser (Coherent Mira 900 with peak power at 7.4 × 1015 W). Here, after photosensitization of RB in a sample
solution containing 1 and RB with the 532 nm, we applied the NIR light. As a result, 1 showed a threefold enhancement in the fluorescence quantum efficiency after 40 min. This enhancement suggests the release of $1\text{O}_2$ by the two-photon absorption mediated photoactivation of $1\text{O}_2$ (Fig. S20) where the singlet oxygen releasing efficiency is also expected to be ca. 1.6% relative to the induced paired-photons. The NIR light-triggered and temporally controlled release of $1\text{O}_2$ provides a promising usage of such dyads for site-specific $1\text{O}_2$ delivery for various fields such as chemistry, material science, and biology.

Conclusions

We revealed the unique properties of an anthracene-coumarin donor–acceptor system (1) during the reactions with $1\text{O}_2$. The fluorogenic $1\text{O}_2$ sensing ability of 1 is unlocked by supplying additional energy by low-intensity UV light or low-energy NIR light after the $1\text{O}_2$ trapping to form $1\text{O}_2$. The intermediate $1\text{O}_2$ is stable against $1\text{O}_2$ scavengers and high temperatures in the dark. Unlike the reported anthracene-linked molecules in the endoperoxide form, $1\text{O}_2$ is rather nonfluorescent. We attributed the photo-triggered fluorescence intensity enhancement to the molecular rearrangement and unique intersystem crossing in $1\text{O}_2$. A photo-triggered release of $1\text{O}_2$ was observed by UV/NIR light illumination on the dye molecule in $1\text{O}_2$ with ~50% yield using EPR spectroscopy. DFT calculations support that the unique excited states and the molecular orbitals of $1\text{O}_2$ offer temporal control over $1\text{O}_2$ capturing, storing, releasing, and sensing. The findings in the present study provide valuable information in the photoexcited state engineering to create novel photofunctional molecular sensors. It will also pave the way for the spatially and temporally controlled utilization of $1\text{O}_2$ in broad areas such as $1\text{O}_2$-mediated chemical reactions and photodynamic therapy.
Data availability
All data generated or analysed during this study are included in this published article and its Supplementary Information file.

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V.B. conceived the idea directed the project. D.S., H.Z., R.K. and Y.T. synthesized the molecules and acquired and analyzed the data. D.S., M.H., and Y.K. performed EPR experiments. V.B., D.S. and Y.T. wrote the manuscript. All the authors contributed to the editing of the manuscript. D.S. and Y.T. contributed equally to this work.

Competing interests
The authors declare no competing interests.

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