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Switching of the Triplet Excited State of Rhodamine-C₆₀ Dyads †Fen Wang,^{a,b†} Xiaoneng Cui,^{a,†} Zhangrong Lou,^a Jianzhang Zhao,^{a,*} Ming Bao^a and Xingwei Li^{b,*}

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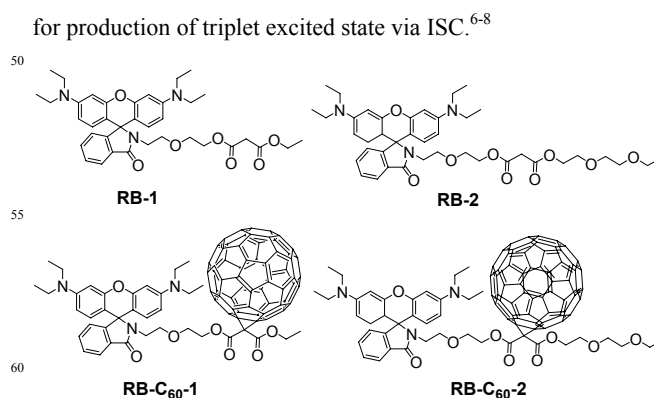
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Acid-switching of the triplet excited state in Rhodamine-C₆₀ dyads was achieved. Rhodamine moiety is acid-activatable visible light-harvesting antenna and C₆₀ is the singlet energy acceptor and the spin converter, and production of triplet state was enhanced in the presence of acid.

Switching of the triplet excited states of organic chromophores is an emerging area owing to its significance in activatable photodynamic therapy (PDT) and molecular devices, but the study on this subject is still in the infancy and very few examples were reported.¹ On the other hand, switching of the *singlet* excited state of organic chromophores has been extensively investigated, and has been widely used in fluorescent molecular probes,² light-harvesting molecular arrays and molecular devices.³ Switching of singlet excited state is usually based on photoinduced electron transfer (PET) or the resonance energy transfer (RET).^{3b} However, there are only few mechanisms for switching of *triplet* excited state of organic chromophores.⁴ Previously amino-containing bromo-azaBodipy was used as pH-activatable photodynamic therapeutic (PDT) reagent.^{1a} Recently molecular logic gates based on modulation of the direction of intramolecular RET of Bodipy dyads were studied, with ¹O₂ production as an output.^{1b,5} However, the strong absorption bands of these compounds in visible region were usually un-switchable.⁶ Furthermore, the triplet excited state switching with organic chromophores has not been studied in detail by nanosecond time-resolved transient difference absorption spectroscopy.^{1a,5} New methodology for switching triplet excited states need to be developed.

Herein we present a new strategy to switch the triplet states of organic chromophores. With our new method, both the triplet excited state and the strong visible light absorption band can be reversibly switched with acid/base. This unprecedented, simultaneous dual mode switching should foster new applications of triplet state switching.

Our method is based on acid-activatable cascade photophysical processes of spiro lactam→opened amide transformation of rhodamine, intramolecular energy transfer (EnT) and intersystem crossing (ISC) in **RB-C₆₀-1** and **RB-C₆₀-2** (Scheme 1).⁶ Using of oligomeric ethylene glycol chain in **RB-C₆₀-2** is to improve the solubility in aqueous solution. Rhodamine moieties are the acid-activatable visible light harvesting antenna, C₆₀ moiety is the singlet energy acceptor and the spin converter,



Scheme 1. Molecular structures of the triplet photosensitizers **RB-1**, **RB-C₆₀-1**, **RB-2** and **RB-C₆₀-2**.

In neutral and basic solution, the rhodamine moiety is in its spiro lactam form, which shows no absorption in visible spectral region.^{3a,9} Thus no EnT and no triplet excited state production are expected when visible light excitation is applied. In acidic solution, however, the rhodamine moiety transforms to its opened amide structure, which shows strong absorption at ca. 550 nm. In this case EnT from the Rhodamine (S₁ state energy level is ca. 2.12 eV) to the C₆₀ part (S₁ state energy level is ca. 1.72 eV) will occur,¹⁰ thereafter the ISC of C₆₀ will produce triplet excited state. Thus in the presence of acid, the rhodamine-C₆₀ dyads will show strong absorption of visible light as well as efficient production of triplet excited state. These features can be reversed upon addition of a base, via the opened amide→spiro lactam transformation of the rhodamine unit.

Firstly the UV/Vis absorption spectra of the compounds were studied. **RB-C₆₀-1** shows no absorption band in visible region (Fig. 1a) and the strong absorption at 340 nm is due to C₆₀ unit. Upon addition of trifluoroacetic acid (TFA), an intense absorption band at 557 nm appeared ($\epsilon = 1.1 \times 10^6 \text{ M}^{-1} \text{ cm}^{-1}$), which is able to be switched off completely by titration with triethylamine (TEA). On the other hand, the fluorescence of **RB-C₆₀-1** can be switched by addition of acid (TFA) or base (triethylamine, TEA) (Fig. 1b). Similar results were also observed for **RB-1**, **RB-2** and **RB-C₆₀-2** (see ESI†, Fig. S16 and S17). The weak fluorescence of the dyads in presence of acid is due to the efficient EnT from the rhodamine moiety to the C₆₀ moiety, which quenches the singlet excited state of rhodamine.

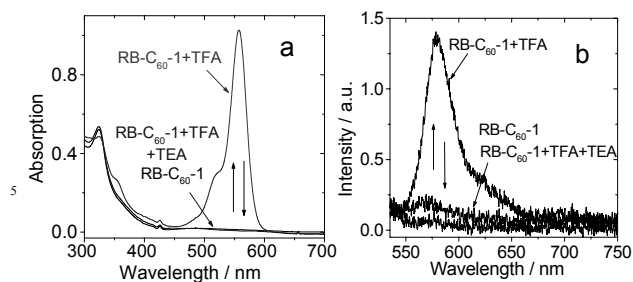


Fig. 1 (a) UV/Vis absorption spectra and (b) fluorescence emission spectra of the compound **RB-C₆₀-1**. $\lambda_{\text{ex}} = 535 \text{ nm}$, $c = 1.0 \times 10^{-5} \text{ M}$ in DCM:CH₃OH (9:1, v/v) with TFA (5.0 mol/L, 50 μL), or TEA (50 μL) added for switching purpose. 20 °C.

To study the EnT between the rhodamine and C₆₀ moiety in the dyads, the fluorescence emission of **RB-1** was compared with that of **RB-C₆₀-1** in the presence of TFA (Fig. 2). **RB-1** gives intense fluorescence emission at 581 nm, but this emission band was quenched in **RB-C₆₀-1** (Fig. 2b), indicating efficient EnT.^{10,11} Similar quenching in **RB-C₆₀-2** was observed (ESI†, Fig. S18). In this case electron transfer (ET) cannot be excluded.^{10,12} Therefore the free energy changes of ET process was calculated to give the ΔG values for **RB-C₆₀-1** and **RB-C₆₀-2** (with RB in opened form) as -0.50 eV and -0.55 eV , respectively (ESI †, Table S1).

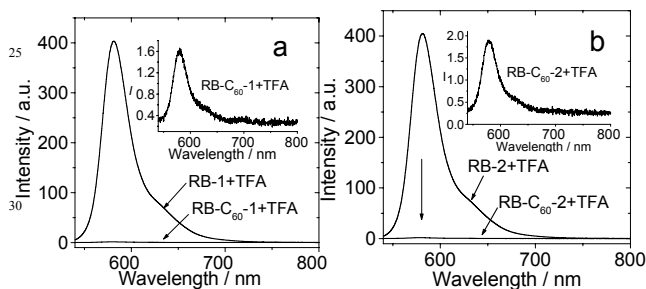


Fig. 2 Fluorescence emission spectra of (a) **RB-1** and **RB-C₆₀-1**, (b) **RB-2** and **RB-C₆₀-2**. $\lambda_{\text{ex}} = 530 \text{ nm}$. In both cases the solution give same absorbance at the excitation wavelength. In DCM/CH₃OH (9:1, v/v) with TFA (5.0 mol/L, 50 μL) added. 20 °C.

A slow kinetic was observed for the spirolactam→opened amide transformation of the rhodamine moiety (ESI†, Fig. S19). The rate constants were determined as $2.61 \times 10^{-3} \text{ min}^{-1}$ and $2.47 \times 10^{-3} \text{ min}^{-1}$ for **RB-C₆₀-1** and **RB-C₆₀-2**, respectively. These values are close to the kinetics of the reference compounds **RB-1** and **RB-2**. Therefore, the spirolactam→opened amide transformation in the dyads were not retarded. The reverse opened amide→spirolactam transformation is fast. Spirolactam ↔ opened amide transformation is widely used in fluorescent molecular design, but the kinetics was rarely studied.^{3a,13}

Photoswitching of the triplet excited states was studied with the nanosecond time-resolved transient difference absorption spectroscopy (Fig. 3).^{10,11} In the absence of TFA, no triplet excited state was detected for **RB-C₆₀-1** upon 557 nm excitation (the time-resolution of the spectrometer is 10 ns). This is due to the extremely weak absorption of C₆₀ moiety in the visible region, thus it is unable to be excited substantially. Spirolactam rhodamine gives no absorption at this wavelength (Fig. 1a).

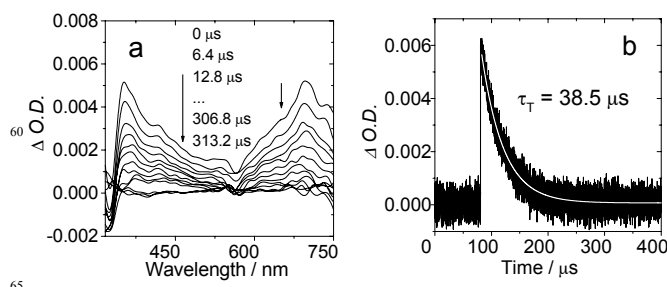


Fig. 3 Nanosecond time-resolved transient difference absorption spectra of **RB-C₆₀-1+TFA** (5.0 mol/L, 50 μL). Transient absorption with different delay times: (a) **RB-C₆₀-1+TFA**, (b) decay trace of **RB-C₆₀-1+TFA** at 450 nm. Excited with OPO nanosecond pulsed laser ($\lambda_{\text{ex}} = 557 \text{ nm}$), $c = 1.0 \times 10^{-5} \text{ M}$, in DCM:CH₃OH (9:1, v/v) after pulsed laser excitation under N₂ atmosphere, 20 °C.

In the presence of TFA, however, transient absorption bands at 720 nm were observed for **RB-C₆₀-1** upon nanosecond pulsed laser excitation at 557 nm (Fig. 3a), which is due to the triplet state of C₆₀.⁸ The lifetime of the triplet state was determined as 38.5 μs . Similar results were observed for **RB-C₆₀-2** (see ESI†, Fig. S21). A control experiment revealed that a much weaker transient signal was observed for a pristine C₆₀ under the same conditions (see ESI †, Fig. S22). Therefore, the production of triplet excited states with the dyads is mainly ascribed to the excitation of the rhodamine moiety and the cascade EnT and ISC. T₁ state of the dyads is localized on C₆₀ moiety rather than the rhodamine moiety, because no ground state bleaching band of rhodamine moiety at 557 nm was observed (Fig. 3a). This triplet state localization profile is due to the lower triplet state energy level of C₆₀ (1.50 eV for C₆₀ versus 1.77 eV for rhodamine).¹⁴

To relay the acid-activated production of triplet excited state of the dyads, photosensitizing of singlet oxygen (¹O₂) by the dyads was studied (Fig. 4). 1,3-Diphenylisobenzofuran (DPBF) was used as a ¹O₂ scavenger (see ESI †, Scheme S2). Production of ¹O₂ can be monitored by following the decrease of the absorption of DPBF at 414 nm. In the absence of TFA, ¹O₂ production capability of **RB-C₆₀-1** is negligible. Upon addition of TFA, however, the ¹O₂ production is substantially enhanced, the Φ_{Δ} value increased to 88.5%. Thus the ¹O₂ production with the dyads is acid-activated. These compounds are useful as candidates for pH-activatable PDT reagents.^{1,4} The photophysical processes of the dyads with and without acid were summarized in Scheme 2.

Intracellular production of the reactive oxygen species (¹O₂) was studied with HeLa cells with confocal microscopy imaging (see ESI†, Fig. S23 and S24). The HeLa cells were stained with DCFH-DA (a singlet oxygen probe). DCFH-DA was nonfluorescent while its oxidized product by ¹O₂ (DCF) emits green fluorescence. The cells give strong green fluorescence upon irradiation (indicating production of ¹O₂) and weak fluorescence without irradiation.

The PDT effect was also studied with the HeLa cells (see ESI †, Fig. S25 and S26). The HeLa cells were incubated with the dyad photosensitizers followed by irradiation with 515–525 nm green LED for 0.5 h. Trypan blue staining of the cells indicated that most of the cells have been killed. In a control experiment, however, the cells were not killed without the photosensitizers or without irradiation. These results show that the triplet photosensitizers can be developed as PDT reagents.

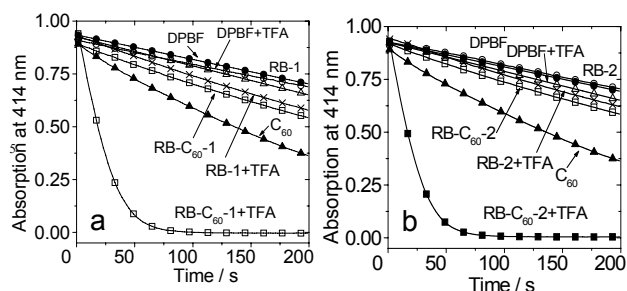
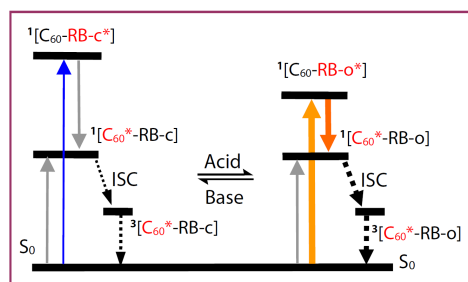


Fig. 4 Kinetics of the singlet oxygen ($^1\text{O}_2$) photosensitizing of the compounds with or without TFA. The decreasing of the absorption of $^1\text{O}_2$ scavenger 1,3-diphenylisobenzofuran (DPBF) was monitored vs. the photoirradiation time. (a) DPBF, DPBF+TFA, **RB-1**, **RB-1+TFA**, **RB-C₆₀-1**, **RB-C₆₀-1+TFA**, **C₆₀**. (b) DPBF, DPBF+TFA, **RB-2**, **RB-2+TFA**, **RB-C₆₀-2**, **RB-C₆₀-2+TFA**, **C₆₀**. $c = 1.0 \times 10^{-5}$ M in DCM : MeOH = 9 : 1 (v/v), 20 °C.



Scheme 2. Jablonski diagram illustrating the photophysical processes involved in the dyads. $[\text{C}_{60}\text{-RB-c}]$ Stands for RB-C_{60} with rhodamine moiety in the spirolactam form. $[\text{C}_{60}\text{-RB-o}]$ Stands for RB-C_{60} with rhodamine moiety in the opened amide form. The localization of the excited state in dyads was designated with red color and asterisk superscript.

In summary, acid-switching of the triplet excited state of organic chromophore, exemplified with rhodamine- C_{60} dyads, were studied. The unique feature of this new triplet state switching profile is that both the strong absorption band in visible spectral region and the triplet excited state can be switched ON and OFF with acid/base. The rhodamine moiety in the dyad is the acid-activatable visible light-harvesting antenna, whereas the C_{60} moiety is the singlet energy acceptor and the spin converter. The acid/base promoted reversible spirolactam/opened amide transformation of rhodamine was used to control the cascade photophysical processes in the dyads. Different from the spirolactam form, the opened amide form of rhodamine shows strong absorption of visible light at 557 nm, thus energy transfer to C_{60} takes place, and the following ISC of C_{60} produces triplet excite state. This unique switching profile offers new approaches for triplet state switching studies, and will be useful for external stimuli-activatable PDT reagents, molecular devices, and the study of photochemistry of organic compounds.

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Notes and references

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[†] Electronic Supplementary Information (ESI) available: molecular structural characterization and other information. See DOI: 10.1039/b000000x/
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