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The dark side of cyclooctatetraene (COT): Photophysics in the singlet states of “self-healing” dyes

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A B S T R A C T

Cyclooctatetraene (COT) attachment to fluorophores (“self-healing” dyes) is known for quenching reactive triplet states *via* triplet-state energy transfer (TET), enhancing photostability. However, COT’s impact on singlet states remains unclear. Quantum calculations reveal that COT induces energy transfer to dark states in deep blue dyes while promoting photoinduced electron transfer (PET) and intersystem crossing (ISC) in visible dyes, potentially compromising brightness and/or photostability. To address this, we propose the use of ΔE descriptor to optimize COT’s effects. Our findings uncover COT’s multifaceted impact. These insights will guide the development of superior triplet state quenchers and photostable dyes.

Photostability plays a pivotal role in numerous applications involving fluorescent dyes, especially in fields like single-molecule studies and super-resolution imaging [1–7]. A highly effective strategy for augmenting photostability involves the direct attachment of cyclooctatetraene (COT) to a fluorophore, thereby quenching its highly reactive triplet state [8,9], improving the overall photo budget [10–14], increasing signal-to-noise ratio [10–12], and minimizing phototoxicity [15–18]. These modified fluorophores are often referred to as “self-healing” dyes [1,19]. However, while COT serves a beneficial role in quenching the triplet states, it remains a subject of investigation whether COT also influences the singlet state photophysics of these self-healing dyes. Understanding these intricacies is critical for harnessing COT’s potential to enhance the photostability of organic dyes and possibly create novel triplet state quenchers (TSQs).

COT has been demonstrated as a highly effective TSQ [2,20]. The triplet states of organic fluorophores exhibited markedly longer lifetimes (typically on the order of µs to ms) compared to singlet states (usually on the order of 0.1–10 nanoseconds). These triplet states can lead to various detrimental effects, such as reactions with oxygen and other reactive oxygen species (ROS), resulting in either recoverable (blinking) or permanent (bleaching) effects on fluorophores. Additionally, triplet states may interact with oxygen, generating highly reactive singlet oxygen or superoxide, causing significant phototoxicity in live cells [21,22]. Effective quenching of triplet states using TSQs, such as COT and its derivatives [23,24], has proven to be an effective strategy in improving fluorophore photostability, enhancing signal-to-noise ratio, and reducing phototoxicity. It is noteworthy that COT exhibits low reactivity towards molecular oxygen [25] and efficiently returns to the ground state [26], making it an “ideal” TSQ.

Extensive research has delved into the photophysics of COT, particularly its ability to quench the triplet states of a wide range of fluorophores *via* triplet state energy transfer (TET) [12,27]. The unique characteristic of COT, allowing non-vertical excitation during S_0-T_1 transition (with a gap of ~0.8 eV [28] to ~1.0 eV [29]), enables it to serve as a “universal” energy acceptor during TET across a broad spectrum, from visible to near-infrared. Theoretical investigations have also rationalized the TET processes from fluorophores to COT, emphasizing the crucial role played by non-vertical excitation in facilitating these processes [30,31]. Surprisingly, less attention has been devoted to understanding singlet state photophysics between fluorophores and COT, with a common belief that COT does not affect fluorophore singlets [20]. Nonetheless, COT’s varying impact on different dyes suggests the existence

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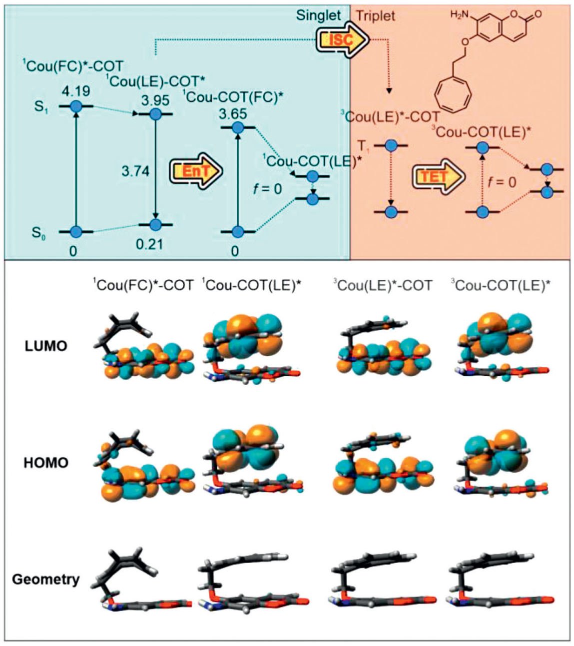

Fig. 1. (Top panel) Proposed mechanism for the photoexcitation and de-excitation of Cou-COT. Jablonski diagram showing the full mechanism including singlet energy transfer (EnT) from coumarin to the COT fragment, ISC, and TET through non-vertical excitation during the S_0-T_1 transition of COT. The inset displays the molecular structure of Cou-COT. (Bottom panel) Frontier molecular orbitals and geometries of Cou-COT in representative states.

of additional photophysics beyond TET [11,12]. Understanding these processes is vital for enhancing fluorophore photostability.

In this study, we employ extensive quantum chemical calculations to unveil two additional photophysical processes within "self-healing" dyes, namely energy transfer to a dark state (ETDS) [32] in deep blue fluorophores and photoinduced electron transfer (PET) [33,34], leading to enhanced intersystem crossing (ISC) in visible fluorophores. Both processes negatively affect fluorophore brightness. We also propose the use of a descriptor ΔE to minimize PET while maximizing COT's positive influence on improving the fluorophore photon budget.

We initiated our investigation by modeling a self-healing dye, Cou-COT, situated in the deep blue spectral range, achieved by directly appending COT to a coumarin derivative (Fig. 1; Figs. S1, S2, and S3a in Supporting information). In this molecular complex, the first ultraviolet–visible (UV–vis) absorption band predominantly arises from the π-π^* transition of the coumarin fragment (the S_1 of coumarin). Below this S_1 state of coumarin, we observed the presence of a low-lying dark state induced by the photoexcitation of the COT fragment (the S_1 of COT). Notably, this dark state exhibits an oscillator strength that approaches zero. However, due to vibronic coupling effects, such dark states can possess weak absorbance characteristics, permitting it to function as an energy acceptor [35].

Upon subsequent geometric relaxation of the coumarin fragment in the excited state, fluorescence emission ensues from its locally excited (LE) state. However, the emission spectrum partially overlaps with the UV–vis absorption spectrum originating from the low-lying dark state of COT. Despite COT's inherently feeble absorbance, the proximity between COT and the fluorophore facilitates highly efficient energy transfer [36]. Our theoretical modeling confirms that COT and coumarin adopt a stacking conformation, with the centroid distance between coumarin and COT measuring a mere 3.544 Å (Fig. S1). Reducing the donor-acceptor distance results in an equivalent enhancement of absorbance [32]. Consequently, the energy can be efficiently transferred from the coumarin fragment to COT. The photoexcited COT, in its S_1 state, undergoes rapid planarization, yielding an exceedingly narrow optical gap that facilitates swift internal conversion and conical intersections [26]. Experimental observations corroborate this behavior, indicating that photoexcited COT does not emit any fluorescence, even at 77 K [37]. This process aligns with the ETDS mechanism [32] and serves as an effective means to quench the fluorescence of coumarin, albeit suboptimal for fluorescence imaging purposes. It is important to note that the triplet state of coumarin can also be quenched *via* triplet energy transfer (TET). However, the ETDS mechanism in singlet states can negatively impact the brightness of self-healing dyes.

Our computational insights are substantiated by experimental data. In a recent study conducted by Lo *et al.*, the attachment of COT to mCP (*N*,*N*′-dicarbazolyl-3,5-benzene) *via* a lengthy aliphatic linker comprising 6 carbon atoms resulted in a reduction in the fluorescence quantum yield of mCP from approximately 0.43 to roughly 0.06 in toluene [37]. We anticipate that shortening this aliphatic linker could further enhance quenching efficiency *via* the ETDS mechanism, akin to observations in tetrazine-based fluorogenic dyes [27].

Continuing our investigation, we turned our attention to modeling a self-healing dye, Cy3-COT, in the visible region (Figs. 2a and b, Fig. S3b in Supporting information). Although TET remains relevant for this dye, our primary focus shifted to the singlet state. The primary UV–vis absorption band of Cy3-COT can be attributed to the π-π^* transition of Cy3. Due to the extended π-conjugation, this S_1 state within the Cy3-COT complex assumes a bright state. Notably, the dark state associated with the COT moiety resides above (the S_3 in Cy3-COT), thereby precluding ETDS from Cy3 to COT.

Upon the geometric relaxation of Cy3, the fluorophore can emit bright fluorescence from the ^{1}Cy3(LE)*-COT state or transition to the triplet state *via* ISC, followed by TET. The stacking conformation in Cy3-COT leads to the reduced local polarity of Cy3. This protective effect of COT minimizes the collisions between Cy3 and other polar solvent molecules. Consequently, this "protective" effect of COT effectively increases the quantum yield of Cy3 by reducing external conversion and other nonradiative decay processes [38].

However, our calculations also unveiled the presence of an electron-transfer (ET) state between Cy3 and COT in the singlet state ($^1[\text{Cy3}^{+\bullet}\text{-COT}^{-\bullet}]$). This state is more stable than the ^{1}Cy3(LE)*-COT state by 0.55 eV, indicating that a considerable proportion of Cy3-COT molecules would undergo PET in the singlet state. In the resulting ET state, ΔE_{ST} is substantially reduced to 0.07 eV. ISC is typically enhanced for the transition from $^1[\text{Cy3}^{+\bullet}\text{-COT}^{-\bullet}]$ to ^{3}Cy3(LE)*-COT (minimizing ΔE_{ST}) than that from ^{1}Cy3(LE)*-COT to ^{3}Cy3(LE)*-COT. In other words, although only a small fraction of Cy3-COT dyes would enter the ET state, ISC would be significantly enhanced by this ET state. It is also possible that the interactions between Cy3-COT introduce additional efficient ISC pathways, such as from ^{1}Cy3(LE)*-COT to $^3[\text{Cy3}^{+\bullet}\text{-COT}^{-\bullet}]$ with a moderate ΔE_{ST} (~0.3 eV) and considerable spin-orbit coupling (SOC) value (8.06 cm^{-1}, Fig. 2a). The enhanced ISC is less favorable for improving photostability, as the introduction of COT aims to quench the triplet states of organic fluorophores. Paradoxically, adding COT also promotes the formation of the triplet state, potentially compromising COT's effectiveness as a TSQ.

Subsequently, we modeled Cy7-COT, a representative near-infrared (NIR) self-healing dye (Figs. 2c and d). The results for Cy7-COT parallel those of Cy3-COT. Although the ET state ($^1[\text{Cy7}^{+\bullet}\text{-COT}^{-\bullet}]$ remains energetically favorable compared to the LE state (^{1}Cy7(LE)*-COT), the energy driving the formation of the ET state decreases from 0.55 eV in Cy3-COT to 0.34 eV in Cy7-COT. These

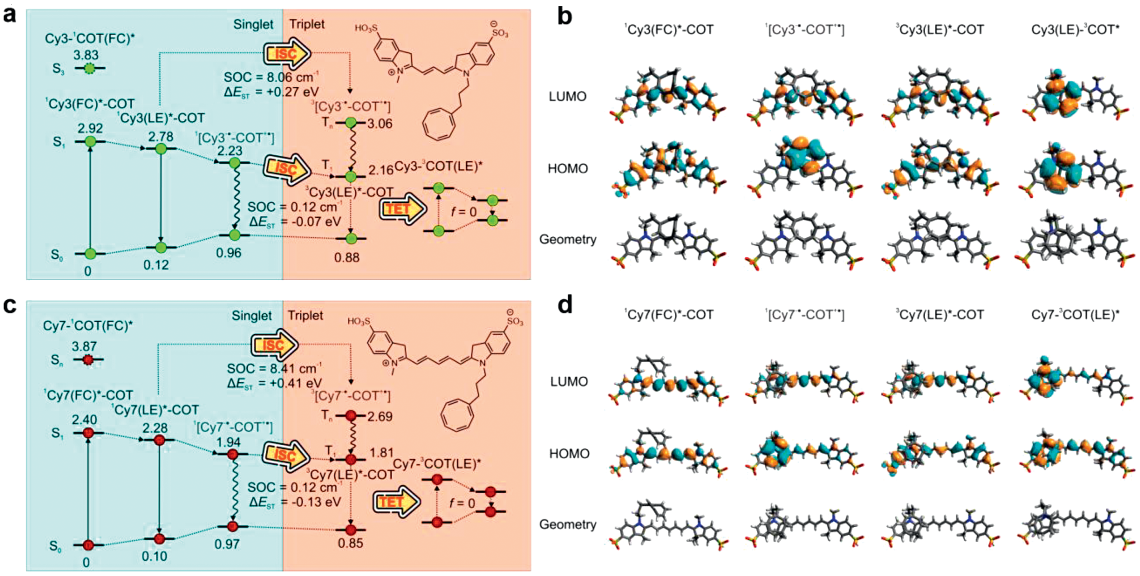

Fig. 2. Proposed mechanism for the photoexcitation and de-excitation of (a) Cy3-COT, and (c) Cy7-COT, involving PET, ISC, and TET. Frontier molecular orbitals and geometries of (b) Cy3-COT and (d) Cy7-COT in representative states. The inset displays the molecular structure of Cy3-COT and Cy7-COT, respectively.

findings suggest that fewer Cy7-COT molecules would enter the ET state, which serves as a conduit for ISC. Similarly, ISC from ^{1}Cy7(LE)*-COT to ^{3}[Cy7$^{+\bullet}$-COT$^{-\bullet}$] also becomes weak with a large ΔE_{ST} (0.41 eV). In other words, in Cy7-COT, COT maximizes its benefits by quenching the triplet states of Cy7 while minimizing the drawbacks associated with enhancing ISC. These phenomena primarily occur because, in long-wavelength fluorophores, PET between the fluorophore and COT tends to be weaker compared to short-wavelength fluorophores (see more discussions in Fig. 3).

Further exploration in the literature supports our computational prediction of PET enhancing ISC, as reported by Bączyński and colleagues, who noted that attaching COT to various rhodamine derivatives indeed enhanced ISC [39]. While Bączyński *et al.* could not explain these experimental observations, our computational modeling illustrates that PET between the fluorophore and COT provides an efficient pathway to expedite ISC. Furthermore, as fluorophores shift towards longer-wavelength regions, this detrimental PET process becomes less likely to occur, consequently rendering the enhancement of photostability more pronounced. For example, Bączyński *et al.* observed a decrease in ISC efficiency when transitioning from short-wavelength rhodamine derivatives to long-wavelength rhodamine derivatives [39]; Blanchard *et al.* also demonstrate that the relative improvement in the photo budget increases from Cy3-COT to Cy5-COT to Cy7-COT [11].

To predict the PET propensity of self-healing dyes, we propose the utilization of the ΔE descriptor (Fig. 3a) [33]. Assuming a relatively uniform and short distance between the fluorophore and COT across various fluorophore-COT complexes, a larger ΔE typically signifies a lower likelihood of PET occurrence. It is important to note that while the classical frontier molecular orbital (FMO) model traditionally suggests that a negative ΔE is imperative for PET to take place, our previous research demonstrates that even a slight positive ΔE can still enable the PET process [33]. This occurs because the FMO model does not fully account for solvent effects, such as dipole-dipole and induced dipole-dipole interactions between the fluorophores in the ET state and the polar solvent molecules surrounding the fluorophore. We have computed ΔE values for a wide range of fluorophores concerning the COT quencher (Figs. S4–S7 and Table S1 in Supporting information), where a larger ΔE indicates a lower likelihood of PET occurrence. In general, long-wavelength fluorophores tend to exhibit larger ΔE values, suggesting a stronger potential for inhibiting PET and enhancing photostability. It is worth noting that for deep blue fluorophores, although PET is energetically feasible, ETDS is likely dominant, as energy transfer is typically more efficient than electron transfer at the same donor-acceptor distance [20,40].

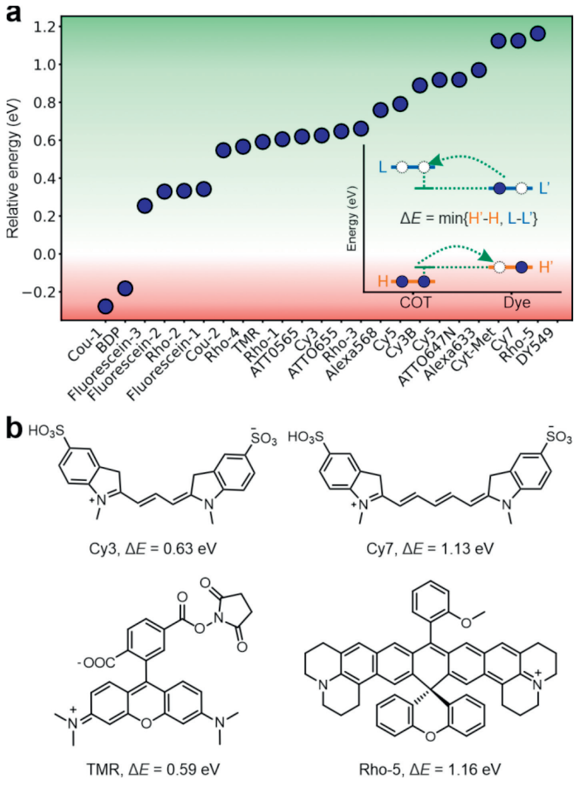

Fig. 3. Evaluating PET between dyes and COT using the ΔE descriptor. (a) The ΔE descriptor is defined as the smaller of the two electronic gaps - between the HOMO of the dye (H') and COT (H, −7.85 eV), or between the LUMO of COT (L, 0.55 eV) and the dye (L'). A higher ΔE indicates less tendency for PET between the dye and COT. The inset shows the calculation method to obtain ΔE. (b) Examples of dyes studied and their corresponding ΔE values. See Supporting information for molecular structures.

We picked up four fluorophores and calculated their respective ΔE values (Fig. 3b). The ΔE value of Cy3 (0.63 eV) is much smaller than that of Cy7 (1.13 eV), indicating the latter has a lower tendency for PET and thus enhanced photostability improvements by COT. This conclusion is consistent with our calculations (Fig. 2) and experimental observations. Similarly, in the two rhodamine derivatives, the ΔE of ECXa (Rho-5) (1.16 eV) [41] is much larger than that of tetramethylrhodamine (TMR) (0.59 eV), indicating that attaching COT to Rho-5 will lead to higher relative improvements in photon budget.

It is essential to recognize that numerous other photophysical processes likely contribute to the singlet state behaviors of self-healing dyes. While our current study predominantly focuses on ETDS and PET, a comprehensive understanding of all such photophysical processes holds significant promise for advancing our knowledge of improving photostability (as well as mitigating phototoxicity) in self-healing dyes.

In summary, our research underscores that COT's influence extends beyond quenching the triplet states of fluorophores *via* TET; it can also actively participate in the singlet state photophysics of self-healing dyes. Specifically, COT can quench fluorescence through the ETDS mechanism in deep blue dyes. Additionally, COT can induce PET between visible fluorophores and itself, creating an ET state that significantly enhances ISC, resulting in a higher population of triplet states in the fluorophore before eventual quenching. The propensity for PET and the associated ISC enhancement tend to decrease as the wavelength of the fluorophores lengthens. We recommend employing the ΔE descriptor as a quick gage of PET tendencies, where a larger ΔE suggests a lower likelihood of PET occurrence. We expect that this study will provide new insights into the development of novel TSQs and strategies for enhancing the photostability of organic fluorophores.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cclet.2023.109227.

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