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H2O2-activated triplet-triplet annihilation upconversion via modulation of the fluorescence quantum yields of the triplet acceptor and the triplet-triplet-energy-transfer efficiency

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Oxidation-activatable triplet-triplet annihilation (TTA) upconversion was achieved with 9,10-bis(diphenylphosphino)-anthracene (BDPPA, nonfluorescent) as activatable triplet
10 **acceptor/emitter, which can be oxidized to BDPPA-O (highly fluorescent) by H2O2 under mild condition, thus TTA upconversion was switched on by H2O2.**

Triplet-triplet annihilation (TTA) upconversion have attracted much attention.[1–14] TTA upconversion is unique because of the
15 requirement of only low power and incoherent photoexcitation (solar light is sufficient), strong absorption of visible light (ε = 80000 – 100000 M^{-1} cm^{-1}), and high upconversion quantum yields (up to 20−30%).[15,16] Moreover, the system is in supramolecular feature and it is readily tunable by using different
20 triplet photosensitizers and triplet acceptors.[2] Recently TTA upconversion has been used in photovoltaics,[17,18] in vivo luminescent bioimaging,[19,20] and light-responsive actuators.[21] However, in order to add more modulability to the application of TTA upconversion, optically or chemically-switchable TTA
25 upconversion system is highly desired. But such TTA upconversion systems are rarely reported.[22,23]

Concerning this aspect, reactive oxygen species (ROS)-activatable TTA upconversion is of in great interest. ROS are endogenous chemical species and they play important roles in the
30 metabolism of cells. For instance, hydrogen peroxide (H2O2) is involved in oxidative stress, and it is a second messenger in normal cellular signal transduction.[24,25] Accordingly, a great amount of fluorescent probes have been developed for detection of H2O2 in living cells.[26–31] However, no H2O2-activated TTA
35 upconversion system was reported.[1,2] Such a TTA upconversion system will be tremendously important for in vivo luminescence bioimaging of the endogenous H2O2 produced in living cells.

Herein we developed a H2O2-activatavle TTA upconversion system, performed under mild conditions (room temperature).
40 9,10-bis(diphenylphosphino)anthracene (**BDPPA**. Scheme 1) was used as H2O2-responsive triplet acceptor/emitter.[21] **BDPPA** is non-fluorescent. Upon oxidation to 9,10-bis(diphenyl-phosphoryl)anthracene (**BDPPA-O**. highly fluorescent. Scheme 1) by H2O2 under room temperature, the TTA upconversion is
45 switched on. **PdTPTBP** (TPTBP = tetraphenyltetrabenzoporphyrin, Scheme 1) was used as red light-excitable triplet photosensitizer.[32,33] Upon addition of H2O2, red-

to-blue upconversion was achieved, which is distinctive to un-aided eyes. This system may be useful for H2O2-responsive
50 luminescence bioimaging with TTA upconversion.

We noted that the **BDPPA-O** was prepared under mild condition by oxidation of **BDPPA** with H2O2.[21,34,35] Thus we envision that a H2O2-activatable TTA upconversion can be developed with **BDPPA**. In the absence of H2O2,
55 **PdTPTBP/BDPPA** gives no upconversion due to the low fluorescence quantum yield of **BDPPA**. In the presence of H2O2, **BDPPA** will be oxidized to **BDPPA-O**, which is highly fluorescent, thus the TTA upconversion will be switched on.
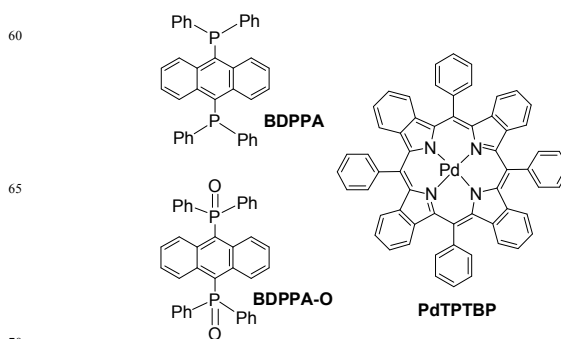

Scheme 1. Triplet photosensitizer **PdTPTBP**, and the triplet acceptors **BDPPA** and **BDPPA-O** used in this study.

Firstly the UV−vis absorption of **BDPPA** and **BDPPA-O** were studied (Fig. 1a). The two compounds give similar
75 absorption bands in the range of 325 − 450 nm. **BDPPA-O** gives slightly red-shifted absorption band than **BDPPA**. In the presence of H2O2, **BDPPA** was oxidized to **BDPPA-O** under room temperature, which is demonstrated by the change of the UV−vis absorption spectra (Fig. 1b).

80 Significant fluorescence enhancement was observed upon oxidation of **BDPPA** (Fig. 2a). **BDPPA** shows negligible fluorescence, whereas **BDPPA-O** is strongly fluorescent (54-fold stronger than **BDPPA**. Optically matched solutions were used).[21,34] We also carried out the *in situ* oxidation of **BDPPA** in
85 the solution, and the fluorescence variation was monitored (Fig. 2b and ESI †, see Fig. S7 for the time course curve of the oxidation). Fast fluorescence enhancement was observed for **BDPPA** in the presence of H2O2 (k = 360 s^{-1}). The fluorescence

switching of **BDPPA** upon oxidation indicates that H_2O_2-activatable TTA upconversion can be achieved with **BDPPA** as the triplet energy acceptor/fluorescence emitter. The photophysical properties of the compounds were listed in Table 1.
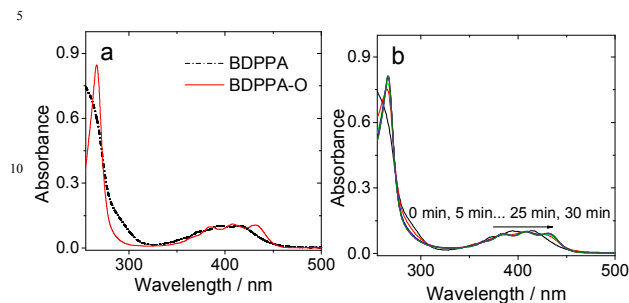
Fig. 1 (a) UV−Vis spectra of **BDPPA** and **BDPPA-O**. (b) UV−Vis spectra of **BDPPA** after addition of 10 μL 30% H_2O_2, $c = 1.0 \times 10^{-5}$ M in MeOH, 20 °C.
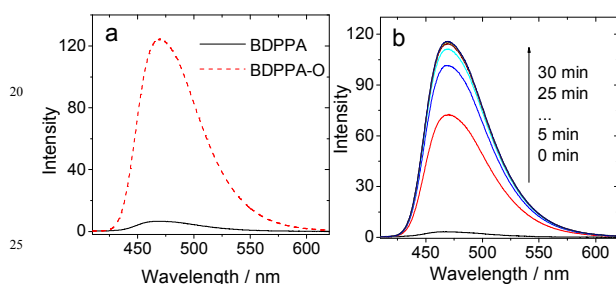

Fig. 2 (a) Fluorescence spectra of **BDPPA** and **BDPPA-O** (excited at 391 nm, optically matched solutions were used, $A = 0.079$ in MeOH); (b) Emission spectra of **BDPPA** after adding 30% H_2O_2 (10 μL). $c = 1.0 \times 10^{-5}$ M in MeOH, 20 °C.

PdTPTBP was used as the triplet photosensitizer for the TTA upconversion (Fig. 3), because the absorption matches with the 635 nm solid state laser.[32,33] **PdTPTBP/BDPPA** gives no TTA upconversion ($\Phi_{UC} = 0\%$). This result is tentatively attributed to the low fluorescence quantum yield of **BDPPA** ($\Phi_F = 1.4\%$). Interestingly, we noted the phosphorescence of **PdTPTBP** was quenched by half, which indicates TTET.[1,2] With **BDPPA-O**, however, strong emission at 470 nm was observed. The emission band is the same to the prompt fluorescence of **BDPPA-O** itself. Excitation of **BDPPA-O** alone at 635 nm under the same condition does not produce any fluorescence emission at 470 nm, (see ESI †, Fig. S8) thus the TTA upconversion with **BDPPA-O** was verified (quantum yield $\Phi_{UC} = 1.8\%$). Interestingly, we found the quenching effect of **BDPPA-O** on the phosphorescence of **PdTPTBP** is much significant than that of **BDPPA**, which indicates more efficient TTET between **PdTPTBP** and **BDPPA-O** than that of **BDPPA** (confirmed in later section).[36]
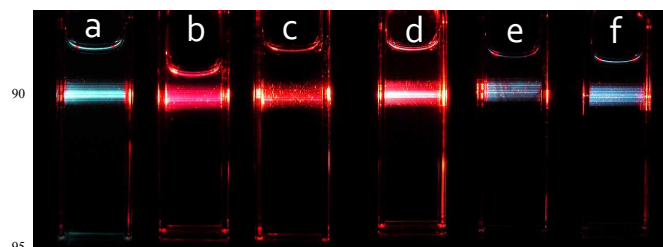
The TTA upconversion with in situ oxidation of **BDPPA** by H_2O_2 was also studied (Fig. 3b). No TTA upconversion was observed for **BDPPA** (with **PdTPTBP** as the triplet photosensitizer). In the presence of H_2O_2, significant upconversion was observed (new emission band at 470 nm was observed). Moreover, the quenching of the phosphorescence of **PdTPTBP** was improved upon oxidation of **BDPPA**. These results are in agreement with that observed with **BDPPA** and **BDPPA-O** (Fig. 3a).
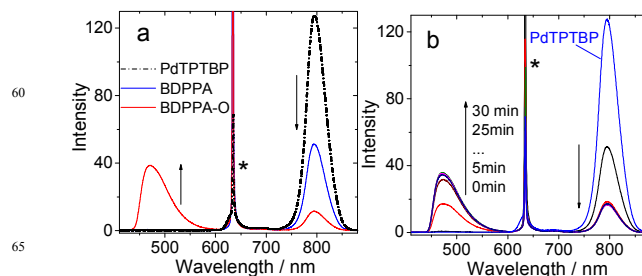
Fig. 3 (a) Upconversion spectra of **BDPPA** and **BDPPA-O** ($c = 7.0 \times 10^{-5}$ M in deaerated MeOH) after addition of **PdTPTBP** ($c = 1.0 \times 10^{-5}$ M) and **PdTPTBP** only ($c = 1.0 \times 10^{-5}$ M in deaerated MeOH), respectively. (b) Upconvertion spectra of mixture **BDPPA** (7.0×10^{-5} M in deaerated MeOH) and **PdTPTBP** ($c = 1.0 \times 10^{-5}$ M) after adding 20 μL 30% H_2O_2. The asterisk in (a) and (b) indicated the scattered laser (635 nm, 10.0 mW, 27.8 mW cm^{-2}). 20 °C.

The red-to-blue upconversion is visible to un-aided eyes (Fig. 4). No upconversion was observed with **BDPPA**, but the phosphorescence of the **PdTPTBP** was quenched (Fig. 4c). In the presence of **BDPPA-O**, strong blue emission was observed (Fig. 4a). In situ oxidation of **BDPPA** with H_2O_2 gives similar results (Fig. 4d–4f). Moreover, we found that $^1O_2$ exerts negligible fluorescence enhancement on BDPPA as compared with that of H_2O_2 (see ESI†, Fig. S11). These results infer that the H_2O_2-actiavated TTA upconversion may be used for in vivo imaging of H_2O_2, probably implemented in the form of polymer film,[37] microcapsule or microbeads.[19,38–41] In this case the H_2O_2 may easily diffuse into the matrix to arouse the oxidation reaction, thus to switch ON the TTA upconversion.


Fig. 4 Upconversion photos of (a) **BDPPA-O** ($c = 7.0 \times 10^{-5}$ M) with **PdTPTBP** ($c = 1.0 \times 10^{-5}$ M), (b) pure **PdTPTBP** ($c = 1.0 \times 10^{-5}$ M) and (c) **BDPPA** ($c = 7.0 \times 10^{-5}$ M) with **PdTPTBP** ($c = 1.0 \times 10^{-5}$ M). The upconversion with in situ oxidation of **BDPPA** ($c = 7.0 \times 10^{-5}$ M) with 20 μL 30% H_2O_2, (d), (e), (f) are the photos taken at 30 s, 4 min, 10 min after addition of H_2O_2, respectively. In deaerated MeOH. Excited with 635 nm laser, power density is 93.3 mW cm^{-2}. 20 °C.

Table 1. Photophysical properties of the compounds

	λ_{abs}[a] / nm	ε[b]	λ_{em} / nm	Φ_F[c] / %	τ_F[d] / ns	τ_T[e] /μs
BDPPA	373/394/414	0.76/1.04/1.05	465	1.4	14.2	−[f]
BDPPA-O	396/418/441	0.96/1.08/1.04	470	75.3	14.4	−[f]
PdTPTBP	628	10.8	796	0	−[f]	61.3

[a] In MeOH ($c = 1.0 \times 10^{-5}$ M). [b] Molar absorption coefficient in toluene. ε: 10^4 M^{-1} cm^{-1}. [c] Fluorescence quantum yield. [d] Fluorescence lifetime. [e] Triplet excited state lifetime. [f] Not applicable.
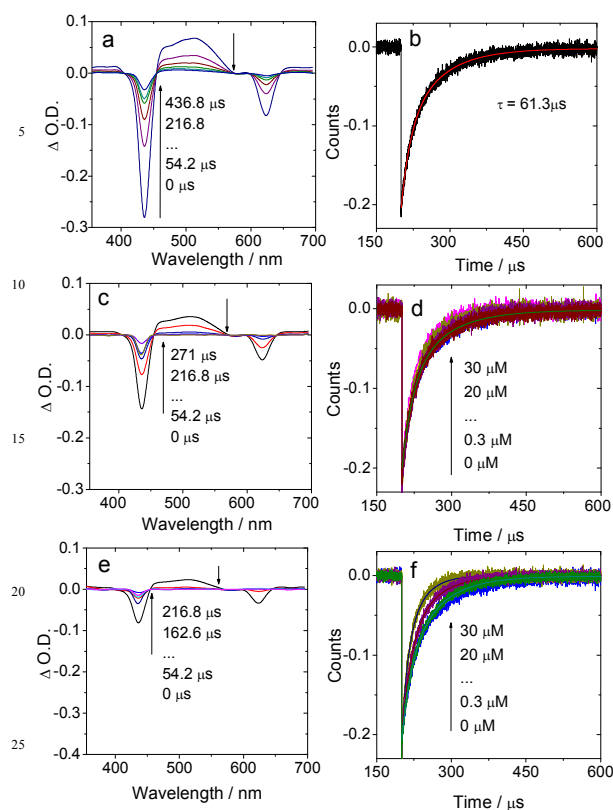
Fig. 5 Nanosecond time-resolved transient absorption spectra of (a) **PdTPTBP** (10 μM), (c) mixture of **PdTPTBP** (10 μM) with **BDPPA** (30 μM) and (e) mixture of **PdTPTBP** (10 μM) with **BDPPA-O** (30 μM); (b), (d) and (f) are the respective decay traces of (a), (c), and (e), with 445 nm nanosecond laser excitation. $c = 1.0 \times 10^{-5}$ M, in deaerated MeOH, 20 °C.

The TTA upconversion indicates that **BDPPA** and **BDPPA-O** exerts different quenching effect on the phosphorescence of **PdTPTBP** (Fig. 3). Thus we studied the TTET in detail with nanosecond transient absorption (TA) spectroscopy (Fig. 5).[42,43] Bleaching bands at 435 nm and 625 nm were observed upon laser excitation for PdTPTBP (Fig. 5a), which is due to depletion of the ground state. Excited state absorption (ESA) in the range of 450 nm – 550 nm were observed, which is attributed to the $T_1 \rightarrow T_n$ transitions. In the presence of **BDPPA**, the optical density (O. D.) values of the bleaching band significantly reduced (Fig. 5c), the triplet state lifetime of **PdTPTBP** was also slightly quenched (Fig. 5d). These changes comfirm the TTET with **BDPPA** as the triplet energy acceptor. In the presence **BDPPA-O**, the O.D. values of the TA spectra of **PdTPTBP** were quenched to larger extent, and the triplet state lifetime of **PdTPTBP** was reduced more significantly (see ESI †, Table S1). These results indicated that **BDPPA-O** is a more efficient quencher than

BDPPA, which is in agreement with the results observed in the TTA upconversion (Fig. 3).[44]

The quantitative comparison of the TTET was performed by using the Stern-Volmer quenching curves (Fig. 6). The figure clearly show that the **BDPPA-O** is more efficient then **BDPPA** as a triplet quencher, for which the Stern-Volmer constants are 6.3×10^4 M^{-1} and 1.5×10^4 M^{-1}, respectively (Table 2).
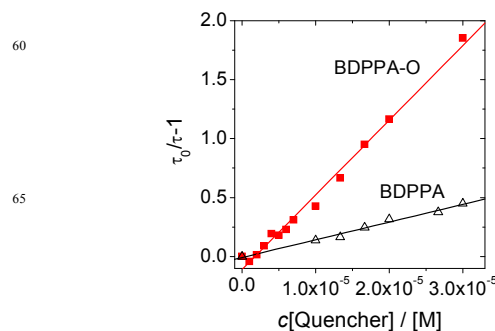
Fig. 6 Stern-Volmer plots of **PdTPTBP** with **BDPPA** and **BDPPA-O** as the triplet acceptor (quencher). $c[$**PdTPTBP**$] = 1.0 \times 10^{-5}$ M in deaerated MeOH, 20 °C.

Since the triplet photosensitizer is the same (**PdTPTBP**), the different TTET efficiency of the triplet acceptors may be due to either the molecular size (diffusion-controlled bimolecular rate constants),[45] or the energy gap of the T_1 state of the photosensitizer and the triple acceptor.[1] Since **BDPPA** and **BDPPA-O** are with similar molecular size thus diffusion-controlled bimolecular collision rate constants are similar (k_0. Table 2).[45] In order to further reveal the different quenching efficiency of **BDPPA** and **BDPPA-O** on the triplet state of **PdTPTBP**, the T_1 state energy levels of the two acceptors were calculated with TD-DFT method, as 1.65 eV and 1.52 eV, respectively. The T_1 state energy level of PdTPTBP is approximated as 1.56 eV with the phosphorescence. Thus, with larger energy gap between the T_1 states of the photosensitizer and acceptor, **BDPPA-O** is a better triplet acceptor (quencher) than **BDPPA**. This different TTET efficiency contributes to the different TTA upconversion efficiency of two triplet acceptors.

In summary, we developed a H$_2$O$_2$-activatable triplet-triplet annihilation upconversion system, with **PdTPTBP** as triplet photosensitizer, and 9,10-bis(diphenylphosphino)anthracene (**BDPPA**) as H$_2$O$_2$-activatable triplet acceptor/emitter. **BDPPA** is non-fluorescent ($\Phi_F = 1.4\%$), but it is able to be feasibly oxidized to 9,10-bis(diphenylphosphoryl)anthracene (**BDPPA-O**) by H$_2$O$_2$,

Table 2. Upconversion-related photophysical parameters. **PdTPTBP** was used as the triplet photosensitizer [a]

	$K_{sv}/10^4$ M^{-1} [b]	$k_0/10^{10}$ M^{-1}s^{-1} [c]	$k_q/10^8$ M^{-1}s^{-1} [d]	f_Q (%)[e]	Φ_{UC} (%)[f]
BDPPA	6.3	3.8	2.5	0.65	0
BDPPA-O	1.5	3.9	10.3	2.63	1.8

[a] In deaerated MeOH. [b] Stern-Volmer quenching constants. [c] Diffusion-controlled bimolecular quenching rate constants. [d] Bimolecular quenching constants. [e] Quenching efficiency. [f] Upconversion yields measured with **M-1** (see ESI †, Scheme. S1) as the standard ($\Phi_L = 10.1\%$ in DCM).

which is strongly fluorescent (Φ_F = 75.3%). Thus TTA upconversion was switched on with H_2O_2. Un−aided eye visible red-to-blue upconversion was observed. Moreover, nanosecond transient absorption spectroscopy demonstrated that **BDPPA-O** is a more efficient triplet quencher than **BDPPA**, for which the Stern-Volmer constant is 6.3×10^4 M^{-1} and 1.5×10^4 M^{-1}, respectively. The different energy transfer may also contribute to the different upconversion efficiencies. TD-DFT calculation indicated that the T_1 state energy level of **BDPPA-O** (1.52 eV) is lower than that of **BDPPA** (1.65 eV). These results are useful for development of H_2O_2-activitable TTA upconversion for in vivo monitoring endogenous H_2O_2 level in living cells or tissues, which can be implemented with microcapsules or microbeads.

Notes and references

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† Electronic Supplementary Information (ESI) available: molecular structural characterization and spectral studies. See DOI: 10.1039/b000000x/

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