## **Supporting Information**

# Organic Nanostructures of Thermally Activated Delayed Fluorescent Emitters with Enhanced Intersystem Crossing as Novel Metal-Free Photosensitizers

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### **Experimental Section**

#### Materials

Both 4,5-bis(carbazol-9-yl)-1,2-dicyanobenzene (2CzPN) and 2,4,5,6-tetrakis(carbazol-9-yl)-1,3dicyanobenzene (4CzIPN) were obtained from Xi'an Polymer Light Technology Corp. while 2,3,5,6tetrakis(3,6-diphenylcarbazol-9-yl)-1,4-dicyanobenzene (4CzTPN-Ph) was synthesized in house as reported previously.<sup>19,20</sup> 1,1-bis[4-[N,N-di(p-tolyl)-amino]phenyl]cyclohexane (TAPC), N,N'-bis(1naphthalenyl)-N,N'-bisphenyl-(1,1'-biphenyl)-4,4'-diamine (NPB) and 3-(4,6-diphenyl-1,3,5-triazin-2-yl)-9-phenyl-9H-carbazole (DPTPCz) which have been reported in our previous work.<sup>24</sup> SOSG was purchased from Life Technologies. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and 9,10-anthracenedipropionicacid (ADPA) were purchased from Sigma-Aldrich. Deionized water with a resistivity higher than 18.4 M $\Omega$ ·cm was collected from an in-line Millipore RiOs/Origin water purification system.

#### Preparation and Characterization of Nanomaterials

2CzPN NRs, 4CzIPN NPs and 4CzTPN-Ph NPs were prepared following methods described in our published work.<sup>29</sup> A, D1, D2, D1:A and D2:A NPs were prepared using the well-documented reprecipitation method. In a typical procedure, 1 mg/mL solution of A, D1, D2, D1:A and D2:A (mass ratio of A and D1/D2 is 1:1) respectively dissolved in THF were prepared. 200  $\mu$ L of the asprepared mixed solution was rapidly dropped into 5 mL of deionized water under vigorous stirring at room temperature for 5 min. Dispersions of the different nanostructures were obtained by further sonication for about 15 min under room temperature to ensure THF evaporation completely. Sizes and morphologies of the nanostructures were investigated using SEM (Philips XL-30 FEG). The SEM samples were prepared by drying a dispersion of the nanostructures on a silicon substrate followed by coating a layer of gold with 2 nm thickness.

#### **Optical Properties Measurements**

UV-vis spectra were recorded using a Cary 50Conc UV-Visible Spectrophotometer. Fluorescence spectra were monitored on a Cary Eclipse Fluorescence Spectrophotometer. Time-dependent

enhancement of fluorescence intensity of SOSG solution experiments were carried out using a xenon lamp (150 W) equipped with a filter passing light from 400 to 700 nm with a power density of 80 mW/cm<sup>2</sup>.

#### <sup>1</sup>O<sub>2</sub> Detection by SOSG

The generation of  ${}^{1}O_{2}$  was detected chemically using singlet oxygen sensor green (SOSG) (Life Technologies) which would increase its green fluorescence emission attributing to an endoperoxide generated in the presence of  ${}^{1}O_{2}$ . The reaction was monitored spectrophotometrically by recording the increase in fluorescence spectra at 525 nm. For the 2CzPN NRs, the 4CzIPN NPs and the 4CzTPN-Ph NPs, a solution of SOSG (100 µL) in milli-Q water (0.165 mM) was mixed with 3 mL of different NR/NP dispersions (40 µg/mL). The samples were irradiated with a xenon lamp (150 W) equipped with a filter passing light of 400 to 700 nm, and their fluorescence spectra at 525 nm were recorded every 5 min over 30 min. Power density: 80 mW/cm<sup>2</sup>. For the D1 NPs, A NPs and D1:A NPs, a solution of SOSG (50 µL) in milli-Q water (0.165 mM) was mixed with 3 mL of different NP dispersions (40 µg/mL). The samples were irradiated with a xenon lamp (150 W) and their fluorescence spectra at 525 nm were recorded every 5 min over 30 min. Power density: 80 mW/cm<sup>2</sup>. For the D1 NPs, A NPs and D1:A NPs, a solution of SOSG (50 µL) in milli-Q water (0.165 mM) was mixed with 3 mL of different NP dispersions (40 µg/mL). The samples were irradiated with a xenon lamp (150 W) and their fluorescence spectra at 525 nm were recorded every 4 min over 20 min. Power density: 80 mW/cm<sup>2</sup>. For the D2 NPs, A NPs and D2:A NPs, a solution of SOSG (50 µL) in milli-Q water (0.165 mM) was mixed with 3 mL of different NP dispersions (30 µg/mL). The samples were irradiated with a xenon lamp (150 W) and their fluorescence spectra at 525 nm were recorded every 4 min over 20 min. Power density: 80 mW/cm<sup>2</sup>.

#### <sup>1</sup>O<sub>2</sub> quantum yield measurements by the disodium salt of ADPA

The generation of  ${}^{1}O_{2}$  was detected chemically using the disodium salt of ADPA (Sigma) which would be bleached to its corresponding endoperoxide upon contact with  ${}^{1}O_{2}$ . Rose bengal was used as the standard photosensitizer ( ${}^{1}O_{2}$  quantum yield  $\Phi_{RB} = 75\%$  in water). The reaction was monitored spectrophotometrically by recording the decrease in optical densities at 378 nm. A solution of ADPA (50 µL) in milli-Q water (1 mg/mL) was mixed with 2 mL of different NPs dispersions (0.03 mg/mL). The samples were irradiated with a xenon lamp (without light-filter) with a power density of 80 mW/cm<sup>2</sup>. To eliminate the inner-filter effect, the absorption maxima of rose bengal and the NPs were adjusted to about 0.2-0.4 OD. The absorption of the disodium salt of ADPA at 378 nm was recorded every 30 sec to obtain the decay rate of the photosensitizing process in a UV-Vis spectrophotometer. The  ${}^{1}O_{2}$  quantum yield of the NP samples were calculated using the following formula according to the ref 1:

#### $\Phi_{\text{sample}} = \Phi_{\text{rose bengal}} * K_{\text{sample}} * A_{\text{rose bengal}} / (K_{\text{rose bengal}} * A_{\text{sample}}),$

where  $K_{sample}$  and  $K_{rose\ bengal}$  are the decomposition rate constants of the disodium salt of ADPA by the TADF based nanostructures and rose bengal, respectively.  $A_{rose\ bengal}$  and  $A_{sample}$  represent the light absorbed by the rose bengal and TADF based nanostructures, respectively, which are determined by integration of the optical absorption bands in the wavelength range 200-700 nm.

Ref 1: Ge, J.; Lan, M. *etc.* A graphene quantum dot photodynamic therapy agent with high singlet oxygen generation. *Nat. Commun.* **2014**, *5*, 4596.

#### In vitro Cytotoxicity by MTT assay

The A549 cells were seeded on 96-well plates in DMEM (with 10% FBS, 1% penicillin/streptomycin). After growing overnight, removed the original medium in each well, then added 200  $\mu$ L of DMEM containing the 2CzPN NRs, 4CzIPN NPs, 4CzTPN-Ph NPs and D1:A NPs to the designated wells. The final concentration of these nanostructures on each plate ranged from 2.5 to 40  $\mu$ g/mL. After 12 h incubation in the dark at 37 °C, the cells incubated with these nanostructures were irradiated for 8 min with white light using a xenon lamp with power density 80 mW/cm<sup>2</sup>. The plates were then incubated at 37 °C in the dark for another 24 h. Then, the original medium in each well was removed. Subsequently, 200  $\mu$ L of DMEM (without FBS) containing 10 % MTT stock solution (5 mg/mL in PBS) were added and incubated for 4 hours. Then the medium containing MTT was completely removed, followed by adding DMSO (200  $\mu$ L) to each well. Cell viabilities were determined by reading the absorbance of the plates at 540 nm using a BioTek Powerwave XS microplate reader. The cells incubated with PBS-supplemented medium represent 100% cell survival. Three replicate wells were run for each concentration.



**Figure S1.** Molecular structure of the organic molecules used in this work. a) 2CzPN, b) 4CzIPN, c) 4CzTPN-Ph, d), D1: TAPC e) D2: NPB, f) A: DPTPCz. Molecules shown in d-f do not have TDAF when used alone.



**Figure S2.** SEM images and photographs (Insets) of the 2CzPN NRs, 4CzIPN and 4CzTPN-Ph NPs dispersed in deionized water respectively.



**Figure S3.** Fluorescence spectra of SOSG solution incubated with deionized water over increasing irradiation time. (Photo-irradiation experiment was carried out using a xenon lamp (150 W) equipped with a filter passing light of 400 to 700 nm. Power density: 80 mW/cm<sup>2</sup>)



**Figure S4**. Normalized absorbance and emission spectra of single free A, D1 and D2 molecules dissolved in THF respectively.



**Figure S5**. Time-dependent enhancement of fluorescence intensity of SOSG solution incubated with a) DI water, b) the D1 NPs, c) the A NPs and d) the D1:A NPs of 40  $\mu$ g/mL over 20 min irradiation time. (Photo-irradiation experiments were carried out using a xenon lamp (150 W). Power density: 80 mW/cm<sup>2</sup>)



**Figure S6**. Time-dependent enhancement of fluorescence intensity of SOSG solution incubated with a) DI water, b) the D2 NPs, c) the A NPs and d) the D2:A NPs of 30  $\mu$ g/mL over 32 min irradiation time. (Photo-irradiation experiment were carried out using a xenon lamp (150 W). Power density: 80 mW/cm<sup>2</sup>)



**Figure S7**. Energy transfer diagrams with exciplexs formation. a) D1:A (TAPC:DPTPCz) and b) D2:A (NPB:DPTPCz).  $S_1^A$ ,  $S_1^{D1}$ ,  $S_1^{D2}$  and  $S_1^{ex}$  are the singlet excited state of the A, D1, D2 and exciplexes, respectively.  $T_1^A$ ,  $T_1^{D1}$ ,  $T_1^{D2}$  and  $T_1^{ex}$  are the triplet excited state of A, D1, D2 and exciplexes, respectively.



**Figure S8.** Singlet oxygen phosphorescence emission spectra from the 2CzPN NRs, 4CzIPN NPs, 4CzTPN-Ph NPs and D1:A NPs dispersed in deionized water respectively. The samples were excited at 380 nm. Phosphorescence emission of  ${}^{1}O_{2}$  was recorded using luminescence spectrometer (FLS920, Edinburgh, equipped with a 450 W broadband Xe lamp) with a silicon filter (1180nm, Iszu Optics, LP1000) located in between the sample and the detector to cut off both the scattering light and stray light having wavelengths shorter than 1180 nm.



Figure S9. Time-dependent bleaching of ADPA caused by  ${}^{1}O_{2}$  generated by a) Rose bengal, b) the 2CzPN NPs, c) the 4CzIPN NPs, d) 4CzTPN-Ph NPs, e) D1:A NPs and f) D2:A NPs dispersed in deionized water respectively.



**Figure S10.** Chemical trapping measurements of the <sup>1</sup>O<sub>2</sub> quantum yield. a) The absorption peak area of rose bengal dissolved in deionized water. b) The absorption peak area of the 2CzPN NPs, the 4CzIPN NPs, 4CzTPN-Ph NPs, D1:A NPs and D2:A NPs dispersed in deionized water respectively. c) The decomposition rate constant of ADPA by RB. d) The decomposition rate constants of ADPA by the TADF based NPs.

The <sup>1</sup>O<sub>2</sub> quantum yields are calculated according to the following formulas:

 $\boldsymbol{\Phi}_{\text{sample}} = \boldsymbol{\Phi}_{\text{rose bengal}} * K_{\text{sample}} * A_{\text{rose bengal}} / (K_{\text{rose bengal}} * A_{\text{sample}}).$ 

(<sup>1</sup>O<sub>2</sub> quantum yield  $\Phi_{RB}$  = 75% in water)

Sample	<i>K</i> (The decomposition rate constant)	A (The absorption peak area)	$\boldsymbol{\Phi}$ ( <sup>1</sup> O <sub>2</sub> quantum yield)
Rose bengal	0.0068	21.7	75 %
2CzPN NPs	0.0017	37.5	10.9 %
4CzIPN NPs	0.0016	37.0	10.3 %
4CzTPN-Ph NPs	0.0018	35.6	12.1 %
D1:A NPs	0.0021	32.6	15.4 %
D2:A NPs	0.00074	38.2	4.6 %

**Table S1.** <sup>1</sup>O<sub>2</sub> quantum yield of the 2CzPN NPs, the 4CzIPN NPs, 4CzTPN-Ph NPs, D1:A NPs and D2:A NPs dispersed in deionized water.