Electronic Supplementary Material (ESI) for New Journal of Chemistry.

Supplementary information

Development of Fluorescent Nanoparticles with Aggregation-Induced Delayed Fluorescence Features, Improved Brightness and Photostability for Living Cells Imaging

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Spectroscopic measurements

The target hybrid DBQ-3PXZ-based NPs sample with an average dye concentration of 0.825 μg/mL was prepared via a typical coprecipitation strategy. The ultraviolet-visible (UV-Vis) absorption spectrum, steady fluorescence spectrum and transient fluorescence spectrum characterizations of the aqueous NPs dispersion sample were performed at room temperature with 3 mL of the sample in a cuvette.

Cell culture

4T1 and bEND.3 cells were cultured in DMEM (Dulbecco's modified Eagle's medium) and supplemented with 10% FBS (fetal bovine serum) and 1% penicillin/streptomycin in an atmosphere of 5% CO_2 and 95% air at 37 ℃.

Cytotoxicity of the hybrid NPs.

4T1 and bEND.3 cells were seeded into 96-well plates at a density of 5×10^3 cells per well in 200 μL DMEM, respectively. Cells were allowed to grow overnight under an atmosphere of 5% CO_2 and 95% air at 37 ℃. The culture media was replaced by the new fresh medium with the various concentration of the NPs sample (0.01, 0.1, 1, 5, 10, 20, 40, 50 μg/mL). After 24 hours, the medium with NPs was replaced with 100 μL of freshly prepared CCK-8 solution (CCK-8: DMEM = 1:9) after washing with DMEM thrice. With further incubation for 3 hours, a microplate reader was used to monitor the absorbance of CCK-8 at 405 nm.

Imaging of the hybrid NPs in 4T1 cells

4T1 cells were seeded on a glass-bottomed dish and allowed to adhere for 24 h. The cells were washed with PBS (pH = 7.4) buffer thrice. Subsequently, the cells were incubated with NPs (10 μg/mL) for 24 hours at 37 °C; the 4T1 cells were rinsed with PBS three times. The 4T1 cells were exposed to 405 nm light and taken a photo at 550-620 nm individually using confocal laser scanning microscopy (CLSM, TCS-SP-5, Leica, Germany).

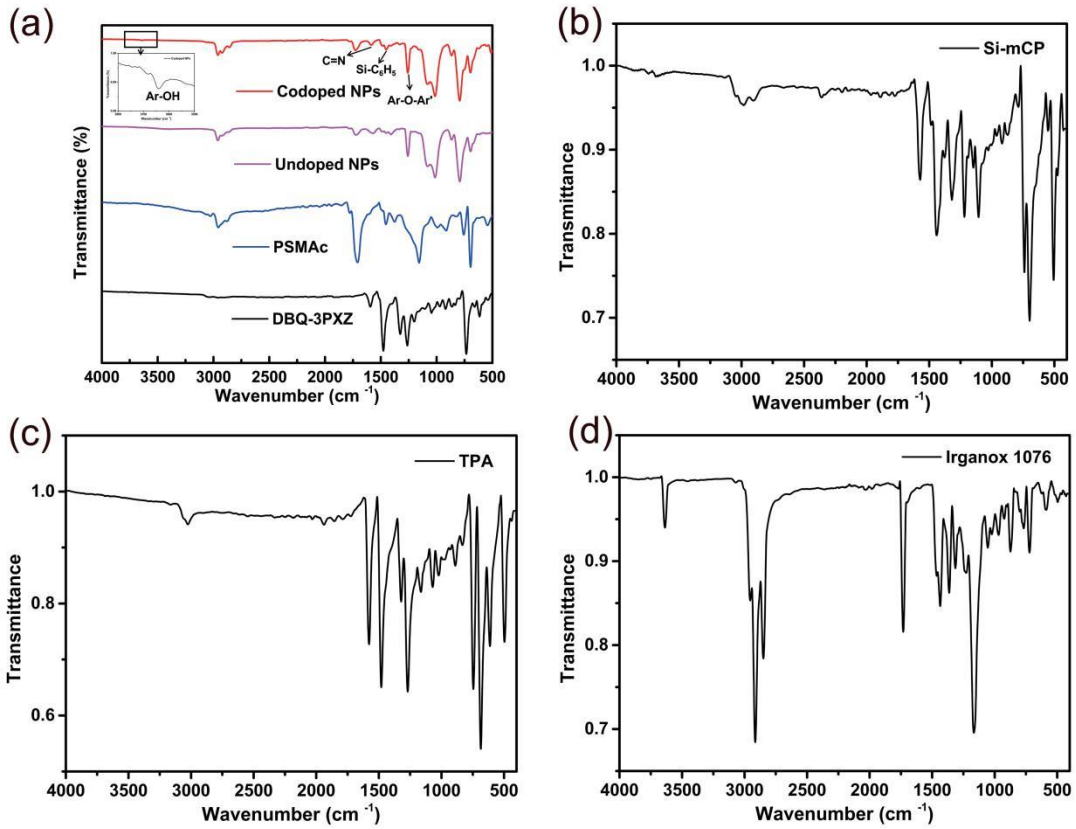

Fig. S1 (a) Normalized FT-IR spectra of DBQ-3PXZ, PSMAc, undoped NPs and codoped NPs samples and of the antifade agents including (b) Si-mCP, (c) TPA and (d) Irganox 1076.

The FT-IR spectra of DBQ-3PXZ, PSAMc, undoped NPs and codoped NPs, which further proves the successful preparation of hybrid NPs. For example, the peaks at 1585 and 1260 cm^{-1} observed in the spectrum of DBQ-3PXZ correspond to stretching vibrational signals of C=N, and Ar-O-Ar', respectively. On the other hand, compared with undoped NPs, the new characteristic peaks of -OH (3640 cm^{-1}) and Si-C_6H_5 (1420 cm^{-1}) in the spectrum of codoped NPs confirm the successful doping of Irganox 1076 and Si-mCP. And the intensity in N-C_6H_5 stretching vibrational signal (1325 cm^{-1}) increased in the spectrum of codoped NPs, which indicates that TPA was doped into the hybrid NPs.

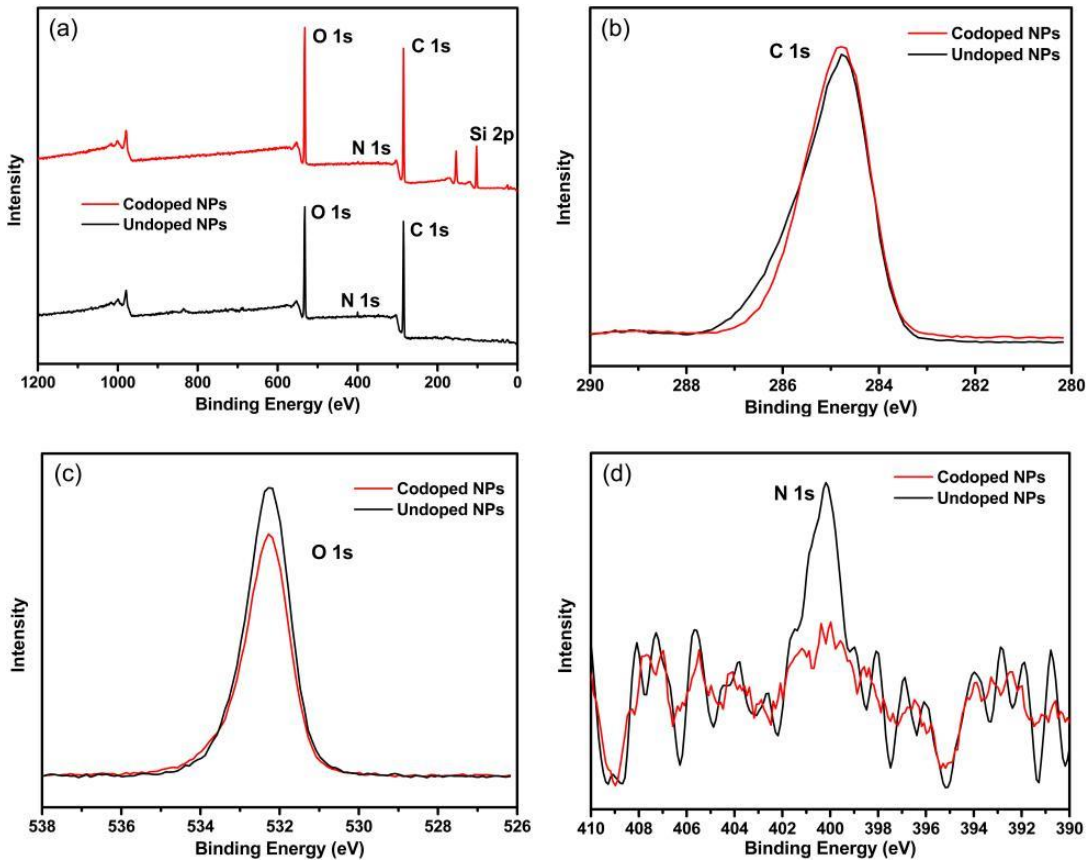

Fig. S2 XPS spectra of undoped NPs and codoped NPs. (a) survey scan ranging from 0 to 1200 eV, (b) C 1s region, (c) O 1s region, (d) N 1s region.

We could observe peaks of C 1s (285 eV), O 1s (532 eV) and N 1s (400 eV), in undoped NPs. The concrete chemical compositions of hybrid NPs are shown in the Table S1. The overall atomic percentage of elements present in undoped NPs was carbon (68.36%), oxygen (30.53%) and nitrogen (1.1%). After incorporating the small-molecular-weight agents into the hybrid NPs, the concentrations of carbon atoms decreased from 68.36% (undoped NPs) to 65.62% (codoped NPs). Alternatively, the concentrations of oxygen atoms decreased to 26.24%, and the concentrations of nitrogen atoms decreased to 0.54%. And there emerged the concentration of silicon atoms (7.6%) originates from Si-mCP. Therefore, the results of XPS spectra could further suggest the successful preparation of codoped NPs.

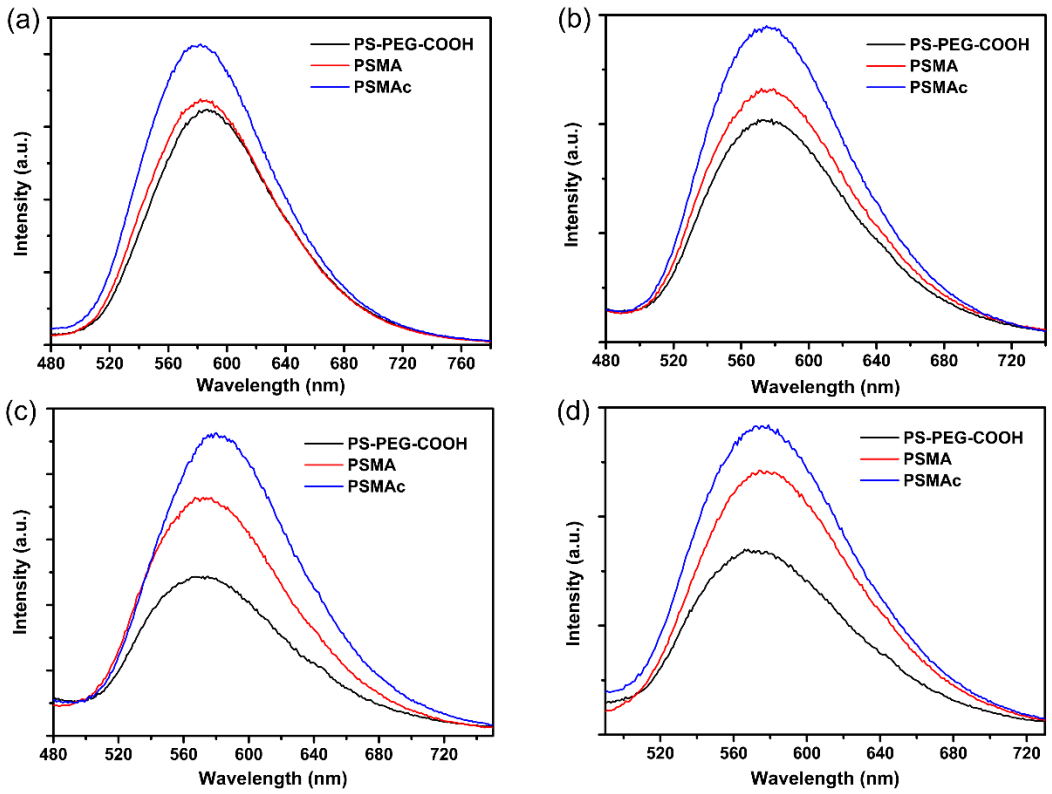

Fig. S3 PL spectra of the DBQ-3PXZ NPs based on three matrices of PS-PEG-COOH, PSMA and PSMAc.

To evaluate the performances of the matrix for hosting the DBQ-3PXZ dye and blocking oxygen, hybrid NPs with the polymer matrix to DBQ-3PXZ dye ratio of 5:1 (a), 10:1 (b), 15:1 (c), 20:1 (d) were prepared and their fluorescence emission performance was evaluated. As compared to NPs with PS-PEG-COOH and PSMA as host matrix, hybrid NPs with PSMAc as the host matrix presented stronger fluorescing ability in the cases of different matrix/dye ratios.

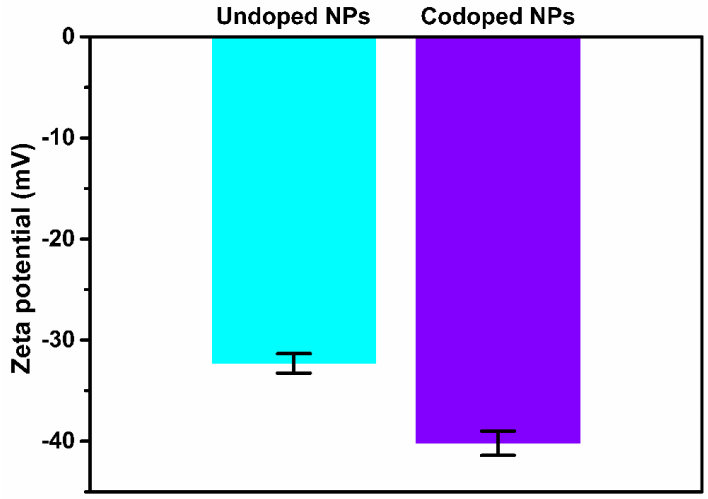

Fig. S4 Zeta potential of hybrid NPs with and without incorporation of antifade agents. Results were expressed as means ± s.d. (n = 3).

The aqueous dispersion sample containing the NPs without dopants reported a zeta potential of approximately -32.3 mV, indicating their negatively charged surface. The hybrid NPs with incorporation of antifade dopants unequivocally displayed a higher zeta potential, -40.2 mV, suggesting the improved colloidal stability of the aqueous NPs sample upon incorporating the small-molecular-weight antifade dopants.

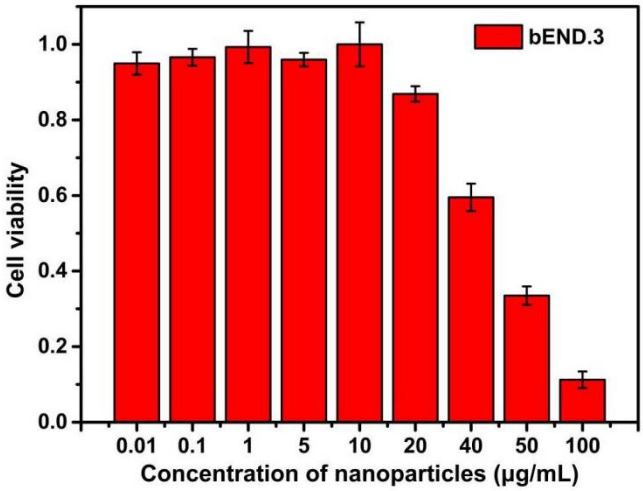

Fig. S5 Cell viability of bEND.3 cells incubated with DBQ-3PXZ-based hybrid NPs at various concentrations from 0.01 μg/mL to 100 μg/mL with incubation time up to 24 h. Results were expressed as means ± s.d.

The bEND.3 cells were selected as the normal cell model for evaluating the biocompatibility of hybrid NPs. Although the cell viability gradually decreased upon

increasing the concentration of the hybrid NPs, more than 86% cells still survived in the case of cells incubation with NPs with concentrations below 20 μg/mL, suggesting the fluorescent NPs developed herein possess lower biological toxicity for normal cells.

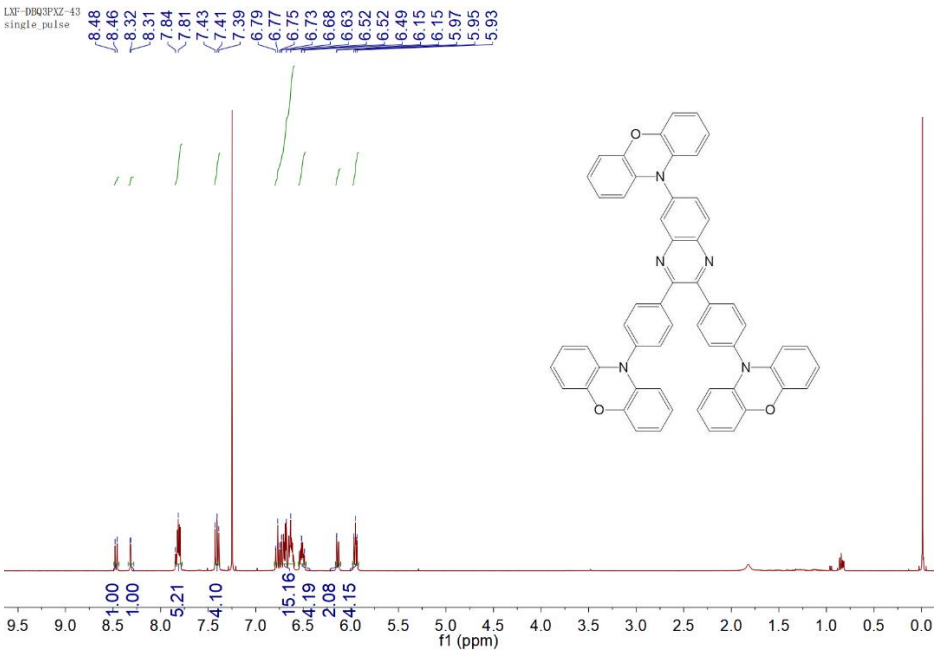

Fig. S6 ^{1}H NMR spectrum of DBQ-3PXZ in $CDCl_3$

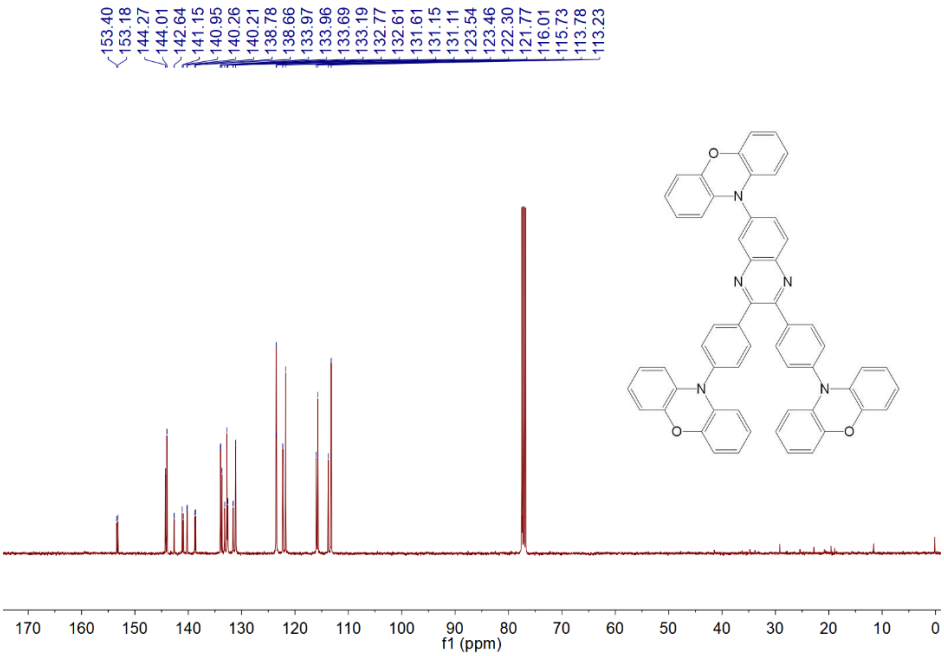

Fig. S7 ^{13}C NMR spectrum of DBQ-3PXZ in $CDCl_3$

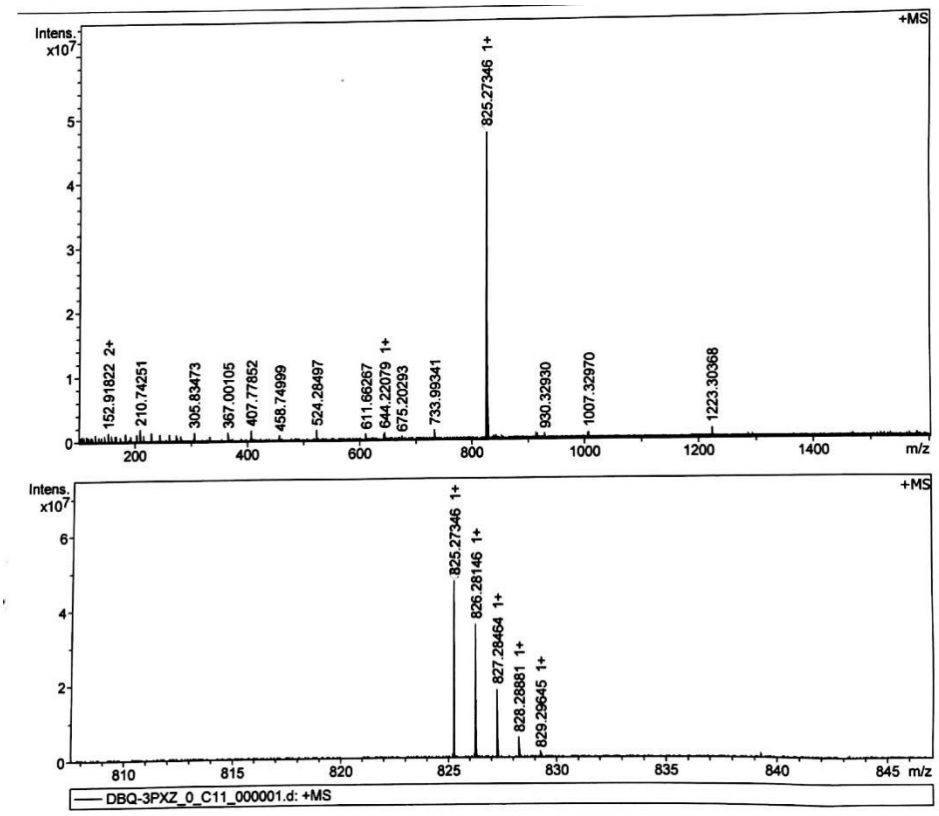

Fig. S8 MALDI-MS characterization results of DBQ-3PXZ

Table S1 Element content (%) of undoped NPs and codoped NPs were calculated based on the XPS analysis

Samples	Si (%)	O (%)	C (%)	N (%)
Undoped NPs	0	30.53	68.36	1.1
Codoped NPs	7.6	26.24	65.62	0.54