Solid-State TICT-Emissive Cruciform: Aggregation-Enhanced Emission, Deep-Red to Near-Infrared Piezochromism and Imaging In Vivo

Yujian Zhang,* Jianxu Zhang, Jiahao Shen, Jingwei Sun, Kai Wang, Zhigang Xie,* Huiwen Gao, and Bo Zou*

1. Introduction

Hydrostatic pressure is a common natural stimulus that can alter the photoluminescence (PL) intensity and color properties through a phenomenon referred to as piezochromism.[1] The practical requirements of flaw detection, mechanical sensors, and deformation detection[2] have promoted the pursuit of piezochromic materials (PCMs) with clear color differences and strong penetrability. However, at present, most reported PCMs exhibit changes in the absorption and/or photoluminescence bands in the visible region (less than 700 nm) [3] and thus generally have poor penetrability. Deep-red/near-infrared (near-IR) fluorescence exhibits longer PL wavelengths and lower energy, which endow it with strong penetrability.[4] Nevertheless, the preparation of near-IR-responsive PCMs remains a significant challenge due to evident PL quenching and a small wavelength shift (faint color difference) in the aggregated state. For the molecular construction of near-IR-responsive fluorophores, one must consider the response sensitivity to improve the color difference. In addition, a strikingly twisted structure should be adopted to acquire high PL quantum yields (PLQY) and avoid the strong intermolecular interactions[5] that give rise to severe fluorescence quenching during the compression process.

Owing to the intriguing photophysical properties of donor (D)–acceptor (A) fluorophores, these systems have drawn considerable attention for versatile applications, such as bio/chem sensors, organic light-emitting diodes (OLEDs), and organic sensors.[6] The most frequently discussed topic with regard to D–A fluorophores is probably the generation of a twisted intramolecular charge transfer (TICT) excited state in polar solvents,[7] which arises from twisting of the DÅ A bond. TICT emission, which is influenced by conformational changes and stabilization/charge separation, is highly sensitive to changes in the external environment. As a result, various types of TICT-based fluorophores that show PL chromism depending on the solvent polarity, temperature, and viscosity in solution have been prepared.[8] For example, a TICT-type pyrene-containing triarylboron molecule,[8b] reported by Yang and co-workers,

Near-infrared piezochromic materials presenting fluorescence responses with clear color differences and good penetrability have important potential applications, but a few such organic compounds are developed. Twisted intramolecular charge transfer (TICT) emission is versatile in solutions, especially for preparing bio/chem-sensing materials due to the excellent sensitivity of the emission to alterations in the external environment. By analogy, the solid-state TICT-emissive chromophores are probably excellent candidates for the environmentally responsive material. Herein, X-shaped π architectures that exhibit solid-state TICT emission are developed, and their luminescent chromism and bioimaging properties are investigated. Initially, the cruciform fluorophore exhibits anomalous aggregation-enhanced emission (AEE) and dual emission due to the existence of a TICT state. Interestingly, TICT emission is observed even in the aggregated state because the spacious environment around the bulky triphenylamine allows for rotation. During the compression process, the TICT-based fluorophore demonstrates deep-red to near-infrared piezochromic behavior with a remarkable redshift (162 nm) and high sensitivity (15.1 nm GPa⁻¹). The bioimaging performance of the TICT-emissive dye suggests its potential utility as a fluorescent probe for biological applications.
exhibited a temperature-dependent green-to-blue fluorescence change in 2-methoxyethyl ether. The accuracy of the thermometer reached as high as 1 °C over a wide temperature range (−50 to 100 °C). Tang and co-workers developed new TICT-emissive aggregation-induced emission luminogens (AIEgens) with a typical propeller-like structure,[8d] showing good selectivity for the detection of Cd(II) in water with a detection limit of 0.88 mmol L−1. Apparently, TICT-based fluorophores in solution exhibit significant PL changes and/or good selectivity upon exposure to external stimuli. By analogy, if such photophysical properties also occur in the aggregated phase, external stimuli may dramatically alter the fluorescence color/intensity. “Therefore, it becomes very critical to design environmentally responsive fluorescent dyes simultaneously possessing high-efficiency emission and TICT process in the condensed state.”[9]

In this study, a new cruciform-shaped fluorophore (DFPA, Figure 1) is designed and synthesized for the development of near-IR PCMs based on the following considerations: bulky triphenylamine (TPA) groups on the horizontal axis provide a large amount of space for molecular rotation, which is beneficial for the formation of the TICT state, while an X-shaped rigid skeleton with highly twisted moieties (TPA and cyanodistyrlybenzene, abbreviated CSB) contributes to high PLQY and weakens the intermolecular interactions under high pressure. As we expected, rotation (θ) of the TPA moieties relative to the central core may occur even in the condensed state, leading to TICT emission. In an in situ high-pressure experiment, the DFPA powder exhibits a prominent shift in the PL peak from 400 nm (bright orange) to 765 nm (dark red). Interestingly, these PL peak wavelengths are linearly related to the hydrostatic pressure with a slope (η, sensitivity) of 15.1 nm GPa−1. This is the first example, to the best of our knowledge, to exhibit near-IR piezochromism based on a TICT-emitting fluorophore in the crystalline state. Moreover, DFPA nanoparticles (DFPA–F127 NPs) show promising potential for in vivo imaging applications.

![Figure 1. The synthetic route of DFPA.](image-url)

2. Photophysical Properties

2.1. Aggregation-Enhanced Emission

As depicted in Figure 1, the X-shaped DFPA molecule was synthesized by Suzuki coupling and Knoevenagel condensation. The molecular structure of DFPA was confirmed by 1H NMR spectroscopy and further established by matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS) (Figure S1, Supporting Information). When illuminated under a UV lamp, dilute tetrahydrofuran (THF) solutions of DFPA emitted no visible light (Figure 2c). However, the crystalline powder of DFPA emitted bright orange light, revealing obvious aggregation-enhanced emission (AEE) features. The PL spectra of the fluorophore in THF and water/THF (f_w) were obtained to examine its AEE properties. In mixtures with low fractions of water (f_w < 50%), weak PL spectra were clearly observed in the range of 425–475 nm, as shown in Figure 2a. At f_w = 60% and 70%, the mixtures revealed dual-peak PL spectra with a new peak generated at 608 nm. When f_w was above 80%, the longer-wavelength peak persisted, while the peak in the lower wavelength region disappeared. At a high f_w of 90%, the solution had a deep-red PL color and exhibited a PLQY as high as 30.4% (Figure 2c). The decreased solubility of DFPA in aqueous mixtures with high water content induced aggregation, which turned the fluorescence on.[10] The formation of lumogen aggregates was confirmed by an upward shift and broadening of the absorption spectrum (Figure 2b). In addition, the morphological observations further suggested that the deep-red fluorescence originated from DFPA aggregates, as depicted in Figure 2d.e. Therefore, the intensified PLQY of DFPA resulted from molecular aggregation, confirming its anticipated AEE properties. In general, the PL spectrum of D–A molecules in the aggregated state reveals a slight blueshift relative to that in polar solvents (e.g., THF) due to the decreased environmental polarity.[11] In contrast, in our case, DFPA aggregates exhibited “anomalous” AEE, and the PL peak was bathochromically shifted by ≈150 nm relative to that of the molecularly dispersed species in THF.

To evaluate the “anomalous” AEE behavior, the UV–vis absorption and PL spectra of DFPA were investigated in various solvents. As shown in Figure S2 (Supporting Information), the X-shaped molecule exhibited two distinct absorption peaks at ≈340 and 460 nm in several solvents. Correspondingly, dual PL peaks were also observed, as shown in Figure S3 (Supporting Information). Interestingly, the dual peaks exhibited different PL lifetimes (Figures S4 and S5, Supporting Information), which meant that two types of excited states existed in solution. The high-energy bands ranging from 400 to 490 nm, similar to those of CN–TPA,[12] revealed no obvious intramolecular charge transfer (ICT) emission and changed slightly with increasing solvent polarity. Thus, these PL peaks could be ascribed to the localized π–π* transition of the CSB
moieties. In sharp contrast, the lower-energy bands gradually broadened and exhibited a remarkable redshift from 608 nm in n-hexane to 654 nm in isopropyl ether (IPE). Meanwhile, the PLQY sharply decreased from 42.8% in n-hexane to 28.8% in IPE and 1.8% in THF. These results explicitly demonstrated that the shifting peaks could be assigned to the ICT transition from the donor (TPA) to the acceptor (CSB).[13] Interestingly, the PL peak from the ICT excited state nearly disappeared in THF due to the high solvent polarity. When the solvent polarity was slightly reduced, an ICT emission appeared in the long-wavelength region, as illustrated in Figure S6 (Supporting Information). Therefore, the “anomalous” AEE of the DFPA fluorophore can be interpreted as follows. In polar solvent (e.g., THF), the X-shaped dye molecule exhibits only locally excited (LE) emission because the ICT excited state is quenched. However, in the aggregated state, the local environment is less polar, which boosts the ICT emission.[11] Thus, the tremendous redshift in the PL spectrum is related to the existence of different excited states, namely, the LE state and the ICT state.

2.2. Solid-State Twisted ICT

LE emission of ICT molecules can usually be observed in frozen or structurally restricted environments.[14] In our case, DFPA molecules exhibited emission from the LE state (Figure S3, Supporting Information) in solution. Intriguingly, the PL intensity of the LE state was enhanced relative to that of the ICT state with increasing solvent polarity (Figure S3, Supporting Information). To explore the nature of these excited states, the radiative decay rate constants ($k_r = \frac{\text{PLQY}}{\tau}$, where $\tau$ is the PL lifetime) of DFPA were evaluated. As presented in Table S1 (Supporting Information), the $k_r$ of the ICT emission decreased gradually from $7.3 \times 10^7$ to $4.8 \times 10^6$ s$^{-1}$ with increasing solvent polarity. These results demonstrated that different excited-state charge-separation processes may result from the rotatable D–A structure. Therefore, the longer-wavelength emission was assumed to be related to the TICT mechanism.[15] Moreover, the ICT emission in the PL spectrum recorded at 77 K was obviously weaker than that recorded at room temperature (R.T.), and LE emission became “dominant” (Figure S7, Supporting Information). This result further supported the hypothesis that dual emission from the LE and TICT states should be possible.[16]

As depicted in Figure S3a (Supporting Information), in n-hexane, DFPA exhibited LE and TICT fluorescence at $\approx 432/452$ and 608 nm, respectively. The PL spectrum of a 0.1% DFPA solid solution (i.e., highly dilute in polymethyl methacrylate) barely changed relative to that of the n-hexane solution in terms of the PL spectra shape and LE peak position (Figure 3a). Moreover, the Stokes shift ($\nu_{\text{LE}} - \nu_{\text{TICT}}$) of the 0.1%
DFPA solid solution \((0.50 \times 10^5 \text{ cm}^{-1})\) was very close to that of the \(n\)-hexane solution \((0.54 \times 10^5 \text{ cm}^{-1})\). These results showed that the “environment” of the DFPA-doped film was similar to that of a low-polarity solvent (e.g., \(n\)-hexane).\[^{13a,17}\] Therefore, this result suggested that a solid solution of DFPA should show TICT emission. To verify this hypothesis, the PL spectra of 1‰ DFPA were measured at various temperatures from 293 to 173 K. As depicted in Figure 3c, the PL intensity ratio \((I_{\text{LE}}/I_{\text{ICT}})\) of LE emission to ICT emission increased slightly with decreasing temperature. These results demonstrated that intramolecular rotation could be thermally blocked by the cooling process. Such behavior was consistent with fluorescence from the TICT mechanism.\[^{8e,16}\] Self-supported films containing 1‰ and 1% w/w DFPA were also prepared. As presented in Figure 3a, the PL peak position of the LE state was nearly unchanged with increasing mass concentration. However, the corresponding intensity of this PL peak gradually weakened up to 1% w/w, and the LE-emissive peak completely disappeared from the PL spectrum, similar to the results observed for the DFPA crystals (Figure 3d). The blend films comprising 0.1‰, 1‰, and 1% w/w DFPA revealed PL lifetimes of 5.3, 7.2, and 8.0 ns (Figure 3b), respectively, demonstrating enhanced intermolecular interactions.\[^{18}\] Thus, we speculated that the quenching of the LE emission was due to intermolecular interactions. Interestingly, the short-wavelength peak from the LE state appeared in the aggregated state (1‰ DFPA and crystalline DFPA) at 77 K, as depicted in Figure 3d. Moreover, the PL intensity ratio between the LE and ICT emissions was clearly higher than that at R.T. These results implied that the TICT process should be responsible for the fluorescence of solid-state DFPA. In other words, intramolecular rotation at the TPA moiety could proceed even in the condensed state.

Considering all of the experimental data together enables us to draw a clear overall picture of the environment-dependent PL processes of DFPA, as depicted in Figure 4a–c. In nonpolar solvent (e.g., toluene), the molecule adopted a twisted conformation \((\theta)\) and showed dual peaks derived from the LE and TICT states (Figure 4a). In a polar solvent (e.g., THF), the TICT peak was completely quenched due to the large nonradiative decay of the TICT state. As a result, the solution revealed LE emission. In solid solution, intramolecular rotation of the TPA unit could occur smoothly. Therefore, dual PL peaks were observed in a low-polarity “environment” (e.g., 0.1‰ TFPA), as illustrated in Figure 4b. Nevertheless, in the crystalline state, the LE emission

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**Figure 3.** a) Steady-state PL spectra and b) time-resolved PL decay curves of a DFPA dye-doped membrane (0.1‰, 1‰, and 1% wt/wt) at R.T. c) Steady-state PL spectra of the doped films (1‰ DFPA) at various temperatures. d) PL spectra of 1‰ DFPA and crystalline DFPA at R.T. and 77 K.
disappeared due to aggregation-caused quenching (ACQ). The DFPA powder exhibited only TICT emission (Figure 4c).

2.3. Theoretical Analysis on TICT

To further understand the TICT process and dual fluorescence, the natural transition orbitals (NTOs) and the associated weights for the first three singlet ($S_0 \rightarrow S_n$, $n = 1, 2,$ and $3$) states of DFPA were determined and are illustrated in Figure 4d. For the $S_0 \rightarrow S_1$ excitation, both the “particle” and the “hole” were distributed on the electron-poor (CSB units) vertical axis, revealing a typical LE transition with a large oscillator strength ($f = 1.3626$). Nevertheless, for the NTOs of the $S_0 \rightarrow S_2$ state, the “hole” density was obviously delocalized over the horizontal axis, while the vertical axis showed whole “particle” density. This transition was mainly dominated by the charge transfer (CT) character. Although the NTOs of the $S_0 \rightarrow S_3$ state also exhibited CT character, such a transition was forbidden as a result of the low oscillator strength ($f = 0.0004$). As shown in Figure 1, steric repulsion existed between several hydrogen atoms located on the central core and TPA unit. This structure strongly suggested that DFPA should possess a significantly twisted conformation along the horizontal axis, even in the solid state. The optimized ground-state geometry showed that the links between the central core and TPA units were twisted with distortion angles ($\theta_{C_1-C_2-N_3-C_4}$) of 64.8° and 61.2° (Figure S9a, Supporting Information). In the excited state, the distortion angles became 79.4° and 54.5° (Figure S9b, Supporting Information), corresponding to a favorable steric distribution for TICT formation.

Figure 4. a–c) The potential energy diagrams and molecular environment-controlled excited-state processes in DFPA. d) The excitation energies and NTOs of the optimized geometric conformation ($\theta_1 = 64.8^\circ$ and 61.2°) determined using the TD/M06-2X/6-31g(d,p) method, where $f$ is the oscillator strength.
In the emission process, the $S_1\rightarrow S_0$ and $S_3\rightarrow S_0$ transitions were clearly allowed and had oscillator strengths of 0.9799 and 0.9689 (Figure S10, Supporting Information), respectively. Moreover, the bulky TPA units offered sufficient space to facilitate a geometric change after photoexcitation, leading to the formation of a TICT state. The results further proved the proposal of dual fluorescence from the LE and TICT states. As shown in Figures S11 and S12 (Supporting Information), the NTOs and oscillator strength of the $S_0\rightarrow S_0$ transition changed slightly with the alteration of the dihedral angle ($\theta$) ($\eta$) (Figures S11 and S12, Supporting Information). Apparently, a slight rotation of the TPA unit had little influence on LE and TICT emission.

2.4. Piezochromic Properties

To gain insight into the TICT process in the crystalline state, in situ high-pressure UV–vis, PL, and infrared (IR) spectroscopy experiments were performed at R.T. via a diamond anvil cell (DAC). A piece of crystal was placed in the hole (diameter: 150 μm) of a T301 steel gasket with silicone oil as a pressure-transmitting medium (PTM). Figure 5a shows that the DFPA crystal emitted bright orange fluorescence with a PLQY as high as 25.2% under ambient pressure. During compression, the PL color of the crystal gradually became deep red, and the corresponding brightness decreased. The PL spectra of the DFPA crystal at various pressures covered the red and near-IR regions. As shown in Figure 5b,c, the PL peak from the TICT excited state gradually redshifted to 634 nm (bright red) at 1.56 GPa and eventually to 765 nm (dark red) at 10.1 GPa, and the PL intensity continuously weakened. The PL intensity at 10.1 GPa was only a hundredth of that at 1 atm. As depicted in Table 1, the TICT-based dye showed near-IR piezochromic properties with a redshift of 165 nm, which is the largest shift achieved among the reported PCMs under high pressure. Interestingly, when the external pressure was fully released, the PL spectrum was restored to the original state, demonstrating the excellent reversibility of the process (Figure S13, Supporting Information). Interestingly, DFPA exhibited ratiometric piezochromic behavior. As shown in Figure 5d, the PL peak from the TICT state was linearly related to the hydrostatic pressure, which implied that the pressure could be quantitatively determined based on the PL peaks. The slope ($\eta$, piezochromic sensitivity) of the PL peak-external pressure plot was calculated by the following equation (Equation (1))

$$\eta = \frac{\lambda_2 - \lambda_1}{P_2 - P_1}$$

(1)

where $\eta$ represents the slope of the curve and $\lambda_1$ and $\lambda_2$ refer to the PL peaks at pressures of $P_1$ and $P_2$, respectively. The piezochromic sensitivity, $\eta$, was estimated to be 15.1 nm GPa$^{-1}$ according to the linear fit (Figure 5d). As depicted in Figure 5e,f, the absorption peaks ascribed to the TICT transition broadened continuously, consistent with the increase in the full width at half maximum of the PL spectra. Moreover, upon increasing the pressure, the absorption sidebands gradually redshifted to longer wavelengths by 150 nm. The corresponding energy gap decreased from 2.23 eV (556 nm) to 1.76 eV (706 nm) (Figure 5g). Thus, the redshift of the TICT emission was considered to be related to molecular planarization.$^{19}$ To further confirm this hypothesis, the excitation energies of molecular configurations with decreasing dihedral angles ($\theta$) were calculated. As depicted in Figure S14 (Supporting Information), rotation of the TPA unit lessens the dihedral angle resulted in a decrease in the $S_1$ excitation energy, which corresponded to a redshift in the TICT bands during the pressurizing process. Thus, the planarity of the molecular conformation might be responsible for the PL redshift.

IR spectra were recorded in the range of 600–3150 cm$^{-1}$ at various pressures to further explore the variation in the molecular structure under different pressures. As depicted in Figure 6a, the wide IR absorption bands observed at 3018 and 2214 cm$^{-1}$ could be readily assigned to the stretching vibrations of aromatic C–H and –C=–N units, respectively. The aromatic C–H and –C≡N stretching frequencies presented clear blueshifts with increasing pressure up to 10.6 GPa (Figure 6c; Figure S15, Supporting Information), which revealed a decrease in the interatomic distances. This change increased the non-radiative vibration processes and intermolecular interactions, leading to a redshift in the PL wavelength and a decrease in the PL intensity.$^{14d,20}$ The stretching vibrations of the phenyl ring at ambient pressure were located at 1590 and 1483 cm$^{-1}$ (Figure 6a). At 1.04 GPa, a weak shoulder peak appeared at 1611 cm$^{-1}$, which was derived from the stretching vibration of vinylene (C=C). With increasing pressure, the intensity of the shoulder peaks clearly increased, as shown in Figure S16 (Supporting Information). The results showed that the extent of electron delocalization increased as a result of the more planar configuration of the CSB moieties.$^{21}$ The =C–H out-of-plane bending mode at 897 cm$^{-1}$ was split at 1.04 GPa, as shown in Figure S17 (Supporting Information). The newly emerged peak (894 cm$^{-1}$) gradually became dominant in the pressure range from 1.04 to 1.99 GPa. In contrast to the blueshifts in the stretching vibrations (e.g., C=C and –C≡N, Figure 6b), the peak position of the =C–H bending vibration barely changed below 3.56 GPa. These results were consistent with the occurrence of conformational planarization proposed above. However, beyond 2.49 GPa, the closer packing of the molecules ultimately restrained the =C–H bending vibration due to the reduced volume. Therefore, these absorption peaks gradually broadened and disappeared. As previously reported, multiple C–H...π and C–H–N interactions existed in the CSB-derivative crystal.$^{6c,17,22}$ Figure S17 (Supporting Information) illustrates that the frequencies of the δ(C–H out-of-plane) bending vibrations at 698 and 753 cm$^{-1}$ simultaneously increased and broadened under high pressure. The multiple C–H...π and C–H–N hydrogen bonds were speculated to deform, revealing a configuration change.$^{21}$

2.5. Imaging In Vivo

The excellent emission properties of DFPA inspired us to investigate its potential application in the imaging of cancer cells and tissues. Stable and dispersed DFPA nanoparticles (DFPA–F127 NPs) were formed in the presence of F127. As shown in Figure S18a (Supporting Information), the DFPA–F127 NPs

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were ≈105 nm in diameter with a polydispersity index (PDI) of 0.16. The transmission electron microscopy (TEM) images display a smooth spherical morphology for the DFPA–F127 NPs. Figure S18b (Supporting Information) shows that the maximum emission of the DFPA–F127 NPs appeared at 604 nm with a large Stokes shift of ≈140 nm, which was favorable for imaging. Photographs of DFPA, F127, and the DFPA–F127 NPs under ambient light and UV light are shown in Figure S18c (Supporting Information), which exhibited similar results to those in Figure S18b (Supporting Information). These results suggest...
that DFPA–F127 NPs were successfully prepared. The DFPA–F127 NPs displayed robust stability in aqueous media for 1 week (Figure S18d, Supporting Information). In addition, the DFPA–F127 NPs showed good biocompatibility (Figure S19a, Supporting Information) toward HeLa cells and excellent photostability upon continuous laser irradiation (Figure S19b, Supporting Information).

To evaluate the performance of the DFPA–F127 NPs in living cell imaging, HeLa cells were cultured with the DFPA–F127 NPs for different time periods. Then, the cell nuclei were dyed with 4,6-diamidino-2-phenylindole (DAPI). As shown in Figure 7, the homogeneous red fluorescence located in the cytoplasm and the fluorescence intensity increased gradually as the incubation time was extended from 1 to 3 h, demonstrating that the DFPA–F127 NPs underwent sustained cellular uptake in HeLa cells. To study the imaging capacity in cancer tissues, DFPA–F127 NPs were intratumorally injected into tumor-bearing BALB/c mice, and the fluorescence was

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<th>Reference</th>
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monitored over 14 days using an in vivo optical imaging system. As shown in Figure 8, the DFPA–F127 NPs showed strong fluorescence at the injection site after injection, and the fluorescence intensity could be readily detected after 14 days, suggesting that the DFPA–F127 NPs had good imaging capacity in vivo.\[24\] The above results confirmed that DFPA–F127 NPs have promising potential for in vivo imaging applications.

3. Conclusion

In summary, an efficient strategy for achieving solid-state TICT emission in X-shaped π architectures has been developed. According to in situ high-pressure experiments, quantum-chemical calculations and dynamic evaluations, the rotation of the TPA moiety toward the central core was confirmed to proceed smoothly due to its spacious environment, resulting in the occurrence of TICT even in the densely packed state. Interestingly, the solid-state TICT emission endowed the DFPA fluorophore with an anomalous AEE effect and near-IR piezochromism. The DFPA crystal quantitatively detected external pressures as high as 10 GPa, exhibiting a large-scale redshift (162 nm), high sensitivity (15.1 nm GPa\(^{-1}\)), and observable color changes (yellow to deep red). Analysis by PL and IR spectroscopies combined with the calculated results illustrated that the piezochromic performance was related to the planarization of the configuration and deformation of the C–H–π and C–H–N hydrogen bonds. Our findings not only deepen the understanding of TICT emission in the aggregated state but also open a new gateway for the design of ratiometric PCMs.

4. Experimental Section

Synthesis of DFPA: The synthetic route to TFPA is depicted in Scheme S1 (Supporting Information). Initially, a Suzuki coupling reaction was undertaken to prepare the key intermediate 4,4″-bis(diphenylamino)-[1,1′,4′,1″-terphenyl]-2,5′-dicarbaldehyde (BTDT), as previously reported.\[17\] Afterward, the Knoevenagel reaction was smoothly performed as follows. First, 0.62 g of 2-(3,5-bis(trifluoromethyl)phenyl)acetonitrile and 0.75 g of BTDT (3 mmol) were added to an ethanol solution (chromatographic pure, 40 mL) at R.T. To this solution, a small amount of NaOMe and THF (40 mL) were added, and the mixture was stirred for 12 h. After the reaction, the mixture was poured into water. The resulting DFPA was filtered and repeatedly washed with EtOH to give a green

Figure 6. a) IR spectra of a DFPA crystal recorded in the range of 600–3150 cm\(^{-1}\) at atmospheric pressure and b,c) corresponding peak positions of the –C–H, –C=N, and –C=C– stretching modes and –C–H bending mode as a function of hydrostatic pressure.

Figure 7. CLSM images of HeLa cells incubated with DFPA–F127 NPs. a,d) DAPI channel. b,e) DFPA–F127 NP channel. c,f) The overlay of panels (a) and (b) and of panels (d) and (e), respectively.
Ocean Optics QE65000 spectrophotometer. In situ IR microspectroscopy of crystalline powders was performed at high pressure on a Bruker Vertex80 FTIR with KBr as the PTM.

**Preparation of Nanoparticles:** DFPA–F127 NPs were prepared using F127 to assemble DFPA in water. In a typical procedure, an F127 solution (200 µL) was first mixed with the DFPA solution (400 µL), and the mixture was added dropwise to 4 mL of Milli-Q water under vigorous stirring at R.T. for 30 min. Then, the solution was dialedyzed against Milli-Q water for 24 h using dialysis bags with a molecular weight cutoff of 3500.

**Biocompatibility of DFPA–F127 NPs in Vitro by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) Assay:** Cells harvested in the logarithmic growth phase were seeded in 96-well plates at a density of 8 \times 10^4 cells per well and incubated in Dulbecco’s modified Eagle medium (DMEM) for 24 h. The medium was then replaced with 200 µL of DMEM containing predetermined concentrations of various NPs, and the cells were incubated for 24 h, after which MTT assays were performed to measure the live cells. Cell viabilities were determined by reading the absorbance of the plates at 490 nm with a microplate reader. Cells incubated with DMEM were used as the control.

**Cell viability (%) = \frac{A_{\text{sample}}}{A_{\text{control}}} \times 100\%** (2)

**Cellular Uptake and Tracking In Vitro:** The cellular uptake of nanoparticles was examined using a confocal laser scanning microscope (CLSM). Cells harvested in the logarithmic growth phase were seeded in 6-well plates at a density of 2.5 \times 10^4 cells per well and incubated in DMEM for 24 h. The medium was then replaced with 2 mL of DMEM containing nanoparticles (5 \times 10^{-6} \text{m} \text{DFPA}), incubated for different periods at 37 °C and further washed three times with phosphate-buffered saline (PBS). For detection by CLSM, the cells were fixed with 4% paraformaldehyde solution for 10 min. After that, the cells were incubated with DAPI for another 5 min to locate the nucleus. Finally, the cells were washed with PBS and observed using CLSM (Zeiss LSM 700).

**In Vivo Imaging in Living Mice:** All animal experiments were performed according to the National Institutes of Health guidelines for the care and use of laboratory animals. HeLa cells were administered by subcutaneous injection into the right flank region of male BALB/c mice. Mouse-bearing tumors were chosen to carry out this study. To detect the imaging capacity, DFPA–F127 NPs (0.5 mg kg^{-1} DFPA) were administered to the mice via intratumoral injection. Then, in vivo imaging was performed under anesthesia using an in vivo imaging system 0, 2, 4, 7, 10, and 14 days post injection. Maestro software was used to remove the mouse background fluorescence.

**Supporting Information**
Supporting Information is available from the Wiley Online Library or from the author.

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Keywords

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