Supporting Information

Efficient dendrimers based on naphthalene indenofluorene for two-photon fluorescent imaging in living cells and tissues

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\textbf{Scheme S1} Synthetic route of DCzNIF and DPaNIF.
Fig. S1 $^1$H NMR spectra of dendrimer CzNIF and AnNIF.
**Fig. S2** MALDI-TOF-MS spectra of compound (a) CzNIF and (b) AnNIF.

**Fig. S3** UV-vis absorption spectra of NIF, DCzNIF, DPaNIF, CzTA, DACz, CzNIF and AnNIF in chloroform.

**Fig. S4** UV-vis absorption spectra of (a) CzNIF and (b) AnNIF in different solvents.
The solvent-polarity-dependent fluorescence spectra can be interpreted in terms of the Lippert-Mataga equation (Equation 1), which expresses the magnitude of the Stokes shift in terms of changes in the molecular dipole moment that occurs concomitantly with electronic excitation.

\[
\nu_a - \nu_f = (\nu_a^0 - \nu_f^0) + \frac{2(\mu_e - \mu_g)^2}{a^2 \hbar c} \epsilon(n) \quad \text{(1)}
\]

Here \(\nu_a\) and \(\nu_f\) are the respective peak absorption and emission energies, respectively, expressed in wavenumbers, \(\epsilon\) is the dielectric constant, \(n\) is the refractive index of the solvent, \(\mu_e\) and \(\mu_g\) correspond, respectively, to the magnitudes of the excited- and ground-state dipole moments, \(\hbar\) is Planck’s constant, \(c\) is the speed of light, and \(a\) is the radius of the solute spherical cavity.

The Lippert plots shown in Fig. S6 are linear, with a correlation coefficient \(R = 0.88\) for CzNIF and 0.94 for AnNIF. Interestingly, the slope of the fitted curve for CzNIF (11667) was much bigger than that of AnNIF (3130.8). According to Lippert-Mataga equation, the excited-state dipole moment, \(\mu_e\), was calculated to be 27.6 D and 13.8 D for CzNIF and AnNIF, respectively. The \(\mu_e\) value of CzNIF was larger than that of 4-(\(N,N\)-dimethylamino) benzonitrile (DMABN), which was a typical charge transfer (CT) molecule with a \(\mu_e\) value of 23 D. The remarkable discrepancy was indicative of the fact that CzNIF was a CT-state-like fluorophore and AnNIF was a local excited (LE) state-dominant fluorophore. This deduction was further confirmed by the photoluminescent quantum yield (\(\Phi_{PL}\)) values of compounds in
demonstrate the typical CT characteristic of CzNIF, whereas AnNIF shows LE state-dominant molecules exhibited relatively lower emission.

Enhanced by up to 3.7 times of CzNIF in DMF (22.6%). Such high result of the enhanced LE component in the emissive state. It is well known that CT-typed molecules in DMF, the high-polarity solvent, AnNIF still obtained high $\Phi_{PL}$ of 84.4%, which was enhanced by up to 3.7 times of CzNIF in DMF (22.6%). Such high $\Phi_{PL}$ values of AnNIF were as result of the enhanced LE component in the emissive state. It is well known that CT-typed molecules exhibited relatively lower $\Phi_{PL}$ values in higher-polarity solvents. The results demonstrate the typical CT characteristic of CzNIF, whereas AnNIF shows LE state-dominant emission.

Fig. S6 Linear correlation of orientation polarization ($f$) of the solvents with the Stokes shift ($v_o - v_f$) for CzNIF and AnNIF.

Fig. S7 2PEF spectra of (a) CzNIF and (b) AnNIF.
**Fig. 58** UV-vis absorption and PL spectra of CzNIF and AnNIF in solid state.

**Fig. 59** DFT calculation result of CzNIF and AnNIF.
The CzNIF and AnNIF with different concentration in chloroform (CHCl₃) were prepared. And their UV-vis absorption spectra were measured as shown in Fig. S11. The absorption intensity at 324 nm for CzNIF and 348 nm for AnNIF versus their concentration curves were shown in Fig. S11(b, d), because the UV-vis absorption profile of CzNIF/AnNIF NPs is close to that of CzNIF/AnNIF (Fig. 4 and Fig. 1), according to the Lambert-Beer law, the absorption intensity is in proportion to the concentration at dilute solution, therefore, the concentration ($c_2$) of CzNIF/AnNIF NPs can be derived based on its absorption intensity at 324 nm for CzNIF NPs and 348 nm for AnNIF NPs. The concentration of CzNIF NPs and AnNIF NPs is 1.2 $\mu$M and 0.8 $\mu$M, respectively.
Fig. S11 UV-vis spectra of (a) CzNIF and (c) AnNIF with different concentrations in CHCl₃ solution and the relationship of concentration and absorption intensity for (b) CzNIF and (d) AnNIF.

Fig. S12 Cell viabilities of Hela cells under the incubation of (a) CzNIF NPs, (b) AnNIF NPs for 6, 24, and 48 h, and (c) fluorescence images of live (green) Hela cells co-stained with calcein-AM and propidium iodide after labled by CzNIF and AnNIF NPs for 6, 24, and 48 h at the concentration of 10 nM.
Fig. S13 Analysis of the DNA content in cell cycle of Hela cells treated with CzNIF NPs for 6h. (a)~(f): the concentration of CzNIF NPs was 0, 2, 4, 6, 8, 10 nM.

Fig. S14 Analysis of the DNA content in cell cycle of Hela cells treated with AnNIF NPs for 6h. (a)~(f): the concentration of AnNIF NPs was 0, 2, 4, 6, 8, 10 nM.
**Fig. S15** One-photon (405 nm) excited CLSM for Hela cells upon treatment with the (a-c) CzNIF NPs and the (d-f) AnNIF NPs (6 nM) for 6 h.

**Fig. S16** (a) the CLSM of Hela cells without nanoparticles and (b) a bar graph between fluorescent intensity and NPs.

**Fig. S17** One-photon fluorescence images of Hela cells under continuous laser scanning for (a) 100 s, (b) 200 s, (c) 300 s, (d) 400 s, (e) 500 s stained with AnNIF NPs, and (f) relative fluorescence intensity ($I/I_0$) of the CzNIF and AnNIF NPs upon continuous laser excitation at 405 nm for 500 s. $I_0$: the initial fluorescence intensity; $I$: the fluorescence intensity of samples at different time points.
Fig. S18 Cell viabilities of BMSCs under incubation of (a) CzNIF NPs and (b) AnNIF NPs for 6, 24, and 48 h.

Fig. S19 2PE fluorescent images of normal liver from mouse without nanoparticles.

Table S1 Optical data of CzNIF and AnNIF in different solvents

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τ_f: the fluorescence lifetime of CzNIF and AnNIF;
Φ_pl: photoluminescent quantum yield of CzNIF and AnNIF;
λ_{abs}^{max}: the maximum absorbance peak;
λ_{PL}^{max}: the maximum emission peak.