Electronic Supplementary Information (ESI)

Constructing NIR silica-cyanine hybrid nanocomposite for bioimaging *in vivo*: a breakthrough in photo-stability and bright fluorescence with large Stokes shift

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Experimental

Materials and apparatus: All reagents and solvents were purchased from commercial sources and are of analytical grade. ^{1}H and ^{13}C NMR in DMSO-d_6 were recorded on a Bruker AvanceIII 400 MHz instruments with tetramethylsilane (TMS) as internal standard. Data for ^{1}H NMR spectra was reported as follows: chemical shift (ppm) and multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet). Data for ^{13}C NMR spectra is reported in ppm. HRMS was performed using a Waters LCT Premier XE spectrometer. UV/Vis spectra were measured with a Varian Cary 500 spectrophotometer (1 cm quartz cell). Emission spectra were measured with Varian Cary Eclipse (1 cm quartz cell). Flash chromatography was conducted by using silica-gel column packages purchased from Qingdao Haiyang Chemical Co. (China). Deionized water was used in the preparation of all samples. The size distribution of nanoparicles (NHs) was evaluated by dynamic light scattering (DLS) with a Nicomp TM 380 ZLS zeta-potential/particle sizer (PSS Nicomp particle size system, USA). Field Emission transmission electron microscopy (TEM) was employed for the morphology on a JEM 2100F electron microscope operated at 200 kV. The photofading of CyN-12, CyN-12@NHs and ICG was induced *in situ* by a continuous wavelength irradiation with Hg/Xe lamp (Hamamatsu, LC8 Lightningcure, 300 W) equipped with cut-off filters. The irradiation power was measured with a photodiode from Ophir (PD300-UV). Absorption changes were monitored by a CCD camera mounted on a spectrometer (Ocean optics). Fluorescence changes were monitored by a Varian Cary Eclipse fluorescence spectrophotometer at fixed intervals.

Synthesis of CyN-12: IR-739 (0.5 g, 0.67 mmol) and dodecylamine (0.375 g, 2.0 mmol) were

dissolved in anhydrous DMF (20 mL). The mixture was stirred at 80-90 °C for 10 h under an argon atmosphere. The solvent was removed under reduced pressure, and then the crude product was purified by silica gel chromatography with dichloromethane/methanol (50:1) to afford the desired product as a deep red solid (180 mg): Yield 30%. ^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ 0.767 (t, 3H, -CH_3), 1.100-1.300 (m, 22H, -CH_2- and N-CH_2-C***H***$_3$), 1.408 (s, 2H, NH-CH_2-CH_2-C***H***$_2$-), 1.777 (t, 4H, NH-CH_2-C***H***$_2$- and cyclohexane-H), 1.900 (s, 12H, -CH_3), 2.550 (t, 4H, cyclohexane-H), 3.750 (d, J = 5.2 Hz, 2H, NH-C***H***$_2$-), 4.140 (d, J = 7.6 Hz, 4H, N-CH_2-), 5.842 (d, J = 12.8 Hz, 2H, alkene-H), 7.401 (t, J = 7.6 Hz, 2H, Ph-H), 7.570 (d, J = 8.8 Hz, 2H, Ph-H), 7.754 (d, J = 12.8 Hz, 2H, alkene-H), 7.988 (d, J = 8.4 Hz, 4H, Ph-H), 8.146 (d, J = 8.8 Hz, 2H, Ph-H), 8.434 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ 11.637, 13.839, 13.900, 21.401, 22.000, 22.059, 24.709, 26.221, 27.622, 28.652, 28.960, 29.008, 30.620, 31.217, 31.261, 48.931, 49.783, 93.561, 110.581, 119.680, 121.555, 123.459, 127.205, 127.912, 129.829, 129.939, 130.356, 130.738, 137.734, 140.106, 167.950, 168.517. Mass spectrometry (ESI-MS, m/z): $[M - I]^+$ calcd for $C_{54}H_{70}N_3$, 760.5570; found, 760.5570.

Synthesis of CyN-12 encapsulated nanocomposites (CyN-12@NHs): The preparation strategy was according to our previous reported procedure.[S1] Briefly, a mixture solution of PS-*b*-PAA (0.015 g), CyN-12 in DMF (10 mL) was prepared in a glass beaker, and stirred in ambient temperature to become homogenous system. Then deionized water (40 mL) was poured into the dye-contained DMF solution under vigorous stirring. The core-shell structured micelles generated by block copolymers were utilized as the matrix, and the saline could be introduced into the system as a stabilizer. The hydrophobic dyes could be easily embedded

into the hydrophobic core during the micelle formation, and the confinement could be further improved by the shell cross-linking with 3-mercaptopropyltrimethoxy silane (MPTMS) at the interface of the core and corona of polymeric micelles. Post-silica coating was done by adding 2 mL of ethanol solution containing MPTMS (150 mg) drop-wise in the presence of 1 mL NH4OH. The reaction was continued for 24 hr. Both DMF and NH4OH were eliminated by dialysis for one day. A blue suspension was obtained, implying that the monodispersed water-solution hybrid nanoparticles were formed and maintained the optical property. The product was collected by centrifugation at 35000 r/min for 1 h, and freeze-dried in vacuum for 24 h. As reported before, the D/P (dye/particle) ratio plays a neglectable role in the fluorescent behavior of the nano-hybrids.[S2] The weight percentage of cyanine dye was about 5%, chosen as the practical proportion without further optimization.

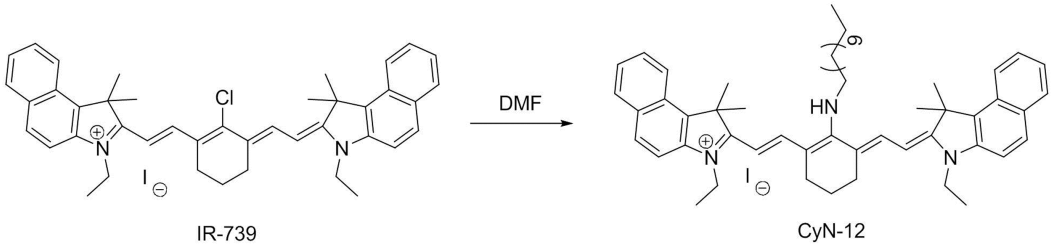
Method for solution preparation of CyN-12, ICG and NaClO: Stock solutions of NaClO were prepared from commercial sodium hypochlorite solution (0.01 M). Stock solutions (0.25 mM) of CyN-12 and ICG were prepared in DMSO. The stock solutions of CyN-12 and ICG were then diluted to 5 μM with corresponding solvent. In the titration experiments, a 2.0 mL solution of CyN-12 (5 μM, the same as ICG) or CyN-12@NHs was poured into a quartz cell and ClO^- solution was added into the quartz cell by using a micro-pipette. The volume of anionic stock solution added was less than 100 μL to remain the concentration of CyN-12 (ICG) and CyN-12@NHs unchanged. Absorptions of CyN-12 and CyN-12@NHs were made almost the same by adjusting the concentration of CyN-12 to 5 μM.

***In-vitro* cytotoxicity test, apoptosis analysis and CLSM observation:** The cell viability was tested by the MTT method. Human hepatocarcinoma HepG2 cells were employed for both the

cytotoxicity tests and *in-vitro* cell imaging. The cells were cultivated at 37 °C in RPMI 1640 with 10% FCS. For the cytotoxicity test, the cells were seeded in a 96-well plate at a density of 8000 cells per well and grown for 24 hr at 37 °C, 5% CO_2 and 95% humidity. After that, freeze-dried CyN-12@NHs were dispersed into the medium with concentrations of 0, 31, 63, 125, 250, 500, 1000 μg/mL, respectively. After 24 h of incubation, 5 mg/mL 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) was dissolved in RPMI 1640 with 10% FCS and incubated for additional 4 h, followed by adding DMSO into each well. Absorption was measured on a microplate reader (ELx800, Bio-Tek) at 650 nm. To evaluated the cell imaging ability, HepG2 cells were seeded in a 1.5 inches imaging plate with an amount of 20000 cells mL^{-1}. The CyN-12@NHs were co-cultivated with HepG2 cells for 12 h at 37 °C, 5% CO_2 and 95% humidity. Before the confocal laser scanning microscopy (A1R, Nikon) observation, the cells were washed 3 times with PBS (pH = 7.4). Emission was collected at 750-800 nm upon excitation at 638 nm.

***In vivo* imaging on a tumor-bearing mouse model:** All animal studies were approved by the Animal Care and Use Committee of Zhejiang University in accordance with the guidelines for the care and use of laboratory animals. 6-8-week-old female BALB/c homozygous athymic nude mice were purchased from the Animal Center of Zhejiang University, and maintained under standard conditions. The nude mice were inoculated with human breast carcinoma BCap37 tumors on their right flanks by injecting 10^6 cells subcutaneously. When the tumors grew up to 10 mm in diameter, the mice were treated with a single intravenous injection via tail vein with CyN-12@NHs at a dose of 0.5 mg/kg and a single intratumoral injection of CyN-12@NHs at a dose of 0.5 mg/kg. Whole body optical imaging was taken 0-24 h after

dye injection using a Kodak In-Vivo FX Professional Imaging System (New Haven, CT) equipped with fluorescent filter sets (excitation/emission, 720/790 nm). The frequency rate for NIR excitation light was 2 μW/cm^2. The camera settings included maximum gain, 2 × 2 binning, 1024 × 1024 pixel resolution, and an exposure time of 20 s. Prior to imaging, mice were anesthetized by intra-abdominal injection of 1% pentobarbital sodium (45 mg/kg). Fluorescence images were co-registered with the anatomical X-ray images using in this system.



Scheme S1. Synthetic route of CyN-12.

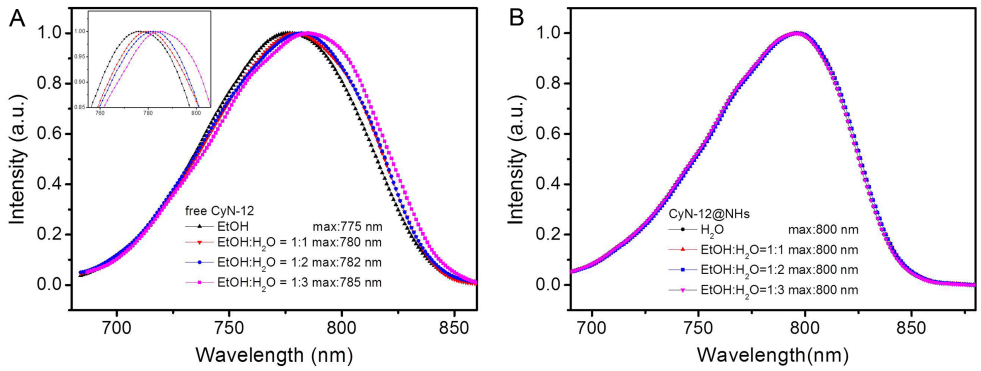


Figure S1. Normalized fluorescence spectra of CyN-12 in free state (A, 5 μM, λ_{ex} = 664 nm) and in encapsulated state (B, λ_{ex} = 690 nm) in a mixture of water/EtOH at different proportion. 2% DMSO was used in the preparation of all solutions of CyN-12.

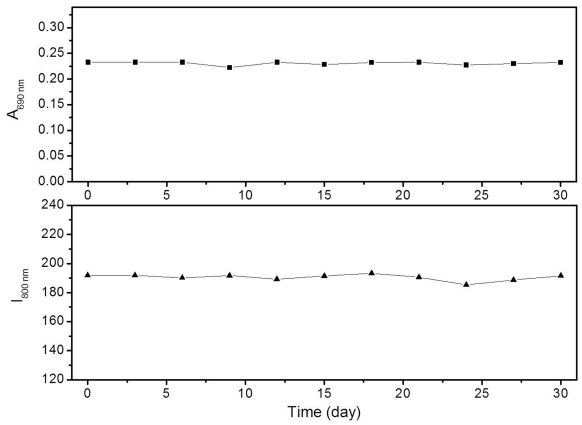


Figure S2. Absorbance at 690 nm and fluorescence intensity at 800 nm (λ_{ex} = 690 nm) in aqueous solution of CyN-12@NHs with aging time from 0 to 30 days under daylight.

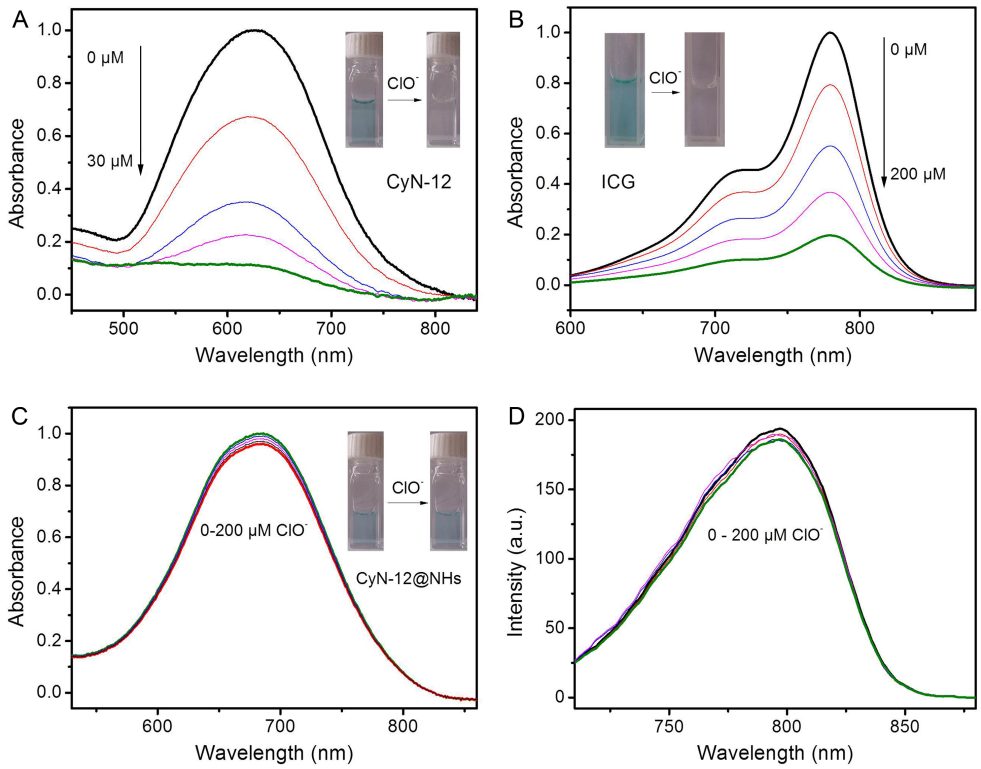


Figure S3. (A) Absorption spectra of CyN-12 (5 μM in water, 2% DMSO) upon titration of NaClO (0, 5, 10, 20, 30 μM), normalizing to the initial absorbance at 635 nm. Inset: Color change observed from solution of CyN-12 upon the addition of NaClO. (B) Absorption spectra of ICG (5 μM in water, 2% DMSO) upon titration of NaClO (0, 50, 100, 150, 200 μM), normalizing to the initial absorbance at 780 nm. Inset: Color change observed from solution of CyN-12@NHs upon the addition of NaClO. (C) Absorption spectra of CyN-12@NHs in aqueous solution upon titration of NaClO (0, 50, 100, 150, 200 μM), normalizing to the initial absorbance at 690 nm. Inset: Color change observed from solution of CyN-12@NHs upon the addition of NaClO. (D) Fluorescence spectra of CyN-12@NHs in aqueous solution upon titration of NaClO (0, 50, 100, 150, 200 μM), λ_{ex} = 690 nm.

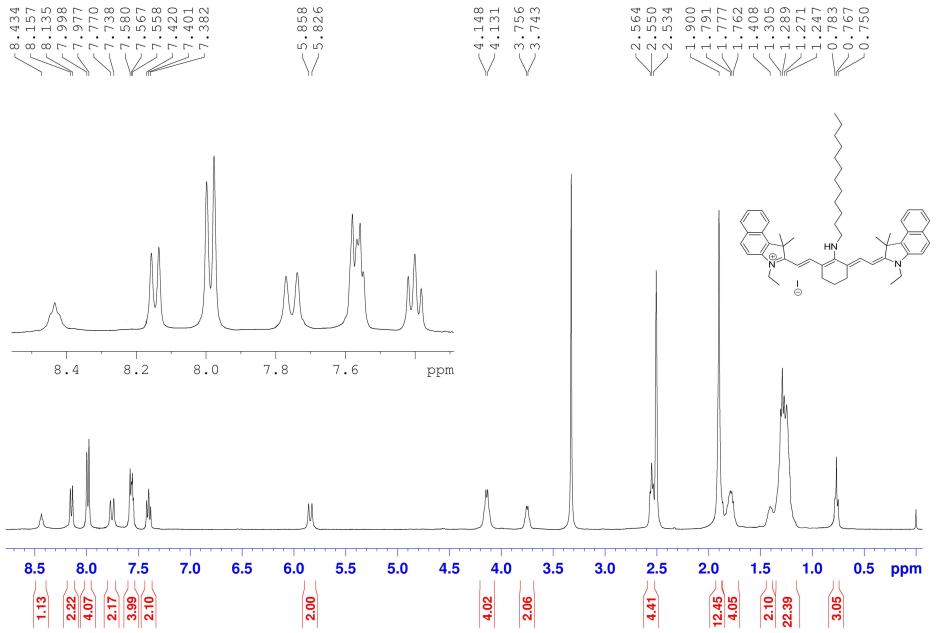


Figure S4. ^{1}H NMR (DMSO-d_6, 400 MHz) spectrum of compound CyN-12.

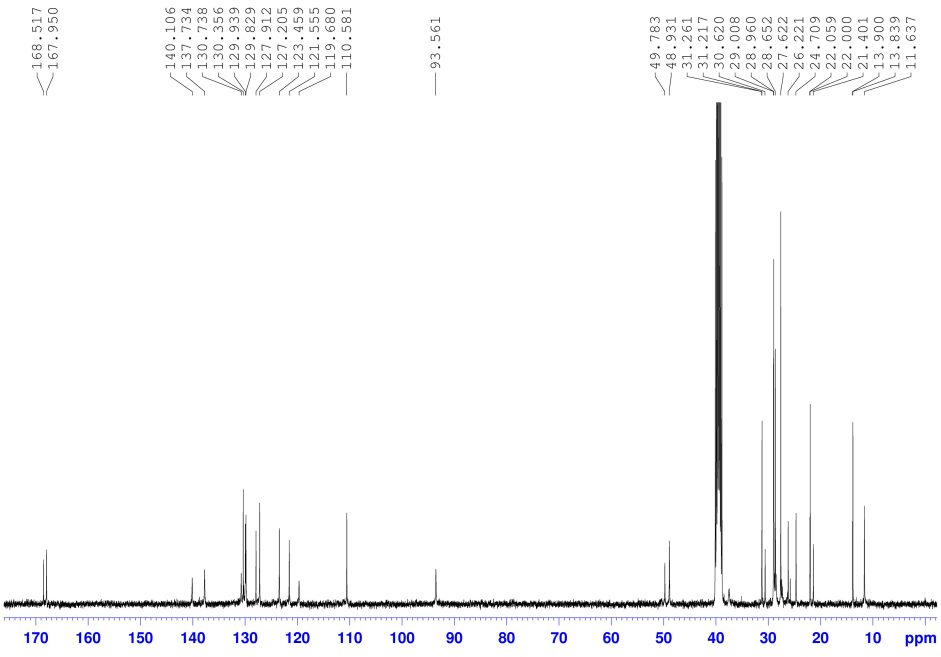


Figure S5. ^{13}C NMR (DMSO-d_6, 100 MHz) spectrum of compound CyN-12.

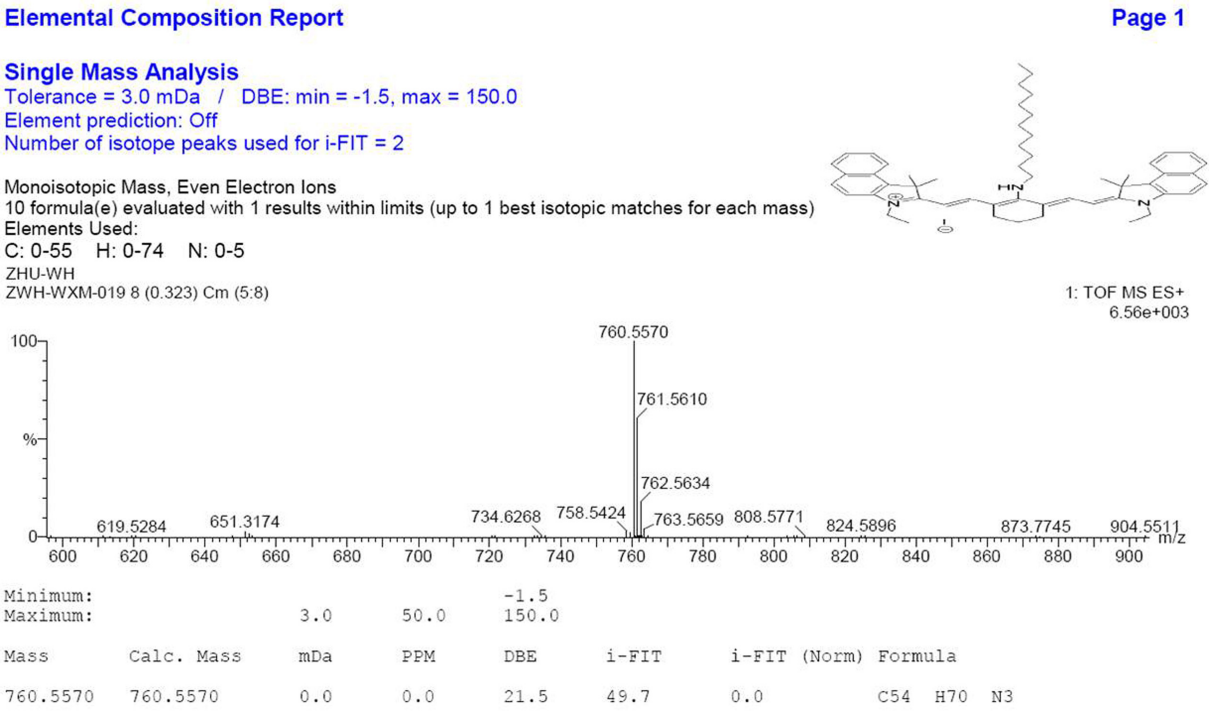


Figure S6. Mass spectrum of compound CyN-12.

References

S1 D. Niu, Y. Li, X. Qiao, L. Li, W. Zhao, H. Chen, Q. Zhao, Z. Ma and J. Shi, *Chem. Commun.*, 2008, **44**, 4463.

S2 S. Chang, X. M. Wu, Y. Li, D. Niu, Z. Ma, W. Zhao, J. Gu, W. Dong, F. Ding, W. H. Zhu and J. L. Shi, *Adv. Healthcare Mater.*, 2012, **1**, 475.