

Supporting Information

FRET-assisted enhanced two photon excited fluorescence in water-miscible doped nanoparticles

Zhenzhen Xu, Qing Liao, Hui Li, Haoli Zhang, Hongbing Fu* and Jiannian Yao

*Beijing National Laboratory for Molecular Science (BNLMS), Institute of Chemistry,
Chinese Academy of Sciences, Beijing 100190, and Graduate University, Chinese
Academy of Sciences, Beijing 100049, College of Chemistry and Chemical
Engineering, Lanzhou University Lanzhou, 730000, P.R. China*

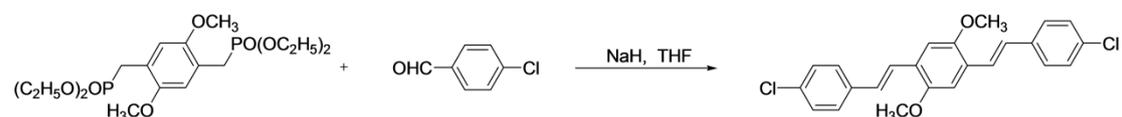
Experimental

1. Materials

The compound used in our work, chlorine-terminated OPV (DCSB) was synthesized as described below. All starting materials and the fluorescent dyes Coumarin 6, rubrene, and DCM were purchased from Sigma-Aldrich and used as received without further purification. Tetrahydrofuran (THF, HPLC grade) was purchased from Beijing Chemical Agent Ltd., China. Ultrapure water with a resistivity of $18.2 \text{ M}\Omega \cdot \text{cm}^{-1}$, produced by using a Milli-Q apparatus (Millipore), were used in all experiments. Alumina membranes with a pore size of 20 nm and polytetrafluoroethylene filters (PTFE, Puradisc 25 TF, 0.1um) were bought from Whatman International Ltd.

2. Synthesis of DCSB

General Procedure :



To the 2,5-bis(methoxy)-1,4-xylene-bis(diethyl phosphonate) (1.00 g, 2.25 mM) and the 4-chlorobenzaldehyde (0.59 g, 4.50 mM) mixture in THF cooled in an ice bath was added 2 eq. NaH (0.22 g, 9.0 mM) in small portions during a 30 min period. The reaction mixture was stirred at room temperature (RT) for 3 h and poured into water. The phase was extracted with CH_2Cl_2 . The pooled organic phases were washed with water, dried over anhydrous MgSO_4 , filtered, and evaporated. The product was separated by flash chromatography on silica gel using CH_2Cl_2 /petroleum ether (1:4) as mobile phase. And DCSB was obtained as a mixture of three isomers. Then the mixed product was dissolved in the minimum amount of a boiling solution containing iodine in toluene (0.1 mM) and refluxed for 12 h. After gentle removal of solvent in *vacuo* the green residue was further purified by flash chromatography on silica gel by means of CH_2Cl_2 /petroleum ether (1:4). Finally a highly fluorescent yellow powder (0.75 g, 1.91 mM) was obtained as the title compound in 85% yield. mp 297°C ; ^1H NMR (300 MHz, CDCl_3) δ 7.65-7.61(m, 10 H), 7.13 (d, $J = 11.6\text{Hz}$, 2H), 7.13 (s, 2 H), 4.05 (s, 6 H, - OCH_3); MS (EI): 392

3. Preparation of the DCSB ONPs

The DCSB ONPs were prepared by the recipitation method. In a typical process, 0.5 mL of 1×10^{-4} M COPV/THF solution or the DCSB@ dopant dyes / THF was added into a 5 mL volumetric flask; then 4.5 mL of water was added quickly with hand-shaking. The concentration of DCSB in the stock solution was fixed at 1.0×10^{-4} M, therefore, 1.0×10^{-5} M in colloidal suspensions. The doping molar ratio between dye components and DCSB matrix in ONPs was adjusted between 0% and 10% by varying the concentration of dyes in the stock solution. The mixture was aged for

about 12 hours. The dispersions of DCSB ONPs into THF:water = 1:9 (v:v) mixture exhibited an off-white turbidity due to the light scattering of particles.

4. Preparation of the DCSB single crystal

The bulk crystals for single crystal X-ray diffraction (SCD) analysis were cultivated by the solvent diffusion method at the liquid-liquid interface between CH₂Cl₂ and MeOH.

5. Measurements

The compound was confirmed by High resolution mass spectroscopy (GCT-MS Micromass, UK) and ¹H-NMR. The morphology of the COPV nanoparticles was characterized by transmission electron microscopy (TEM, JEOL-1011). The X-ray diffraction (XRD) patterns were measured by a D/max 2400 X-ray diffractometer with Cu K α radiation ($\lambda=1.54050$ Å) operated in the 2θ range from 3° to 30°, by using the samples of nanoparticles filtered on the surface of an AAO membrane.

The steady-state absorption spectra were measured on a Shimidazu UV-3600 UV-VIS-NIR spectrophotometer. The stationary fluorescence spectra were performed on a Horiba FluoroMax-4-NIR spectrophotometer equipped with an integrating sphere. The relative fluorescence quantum yields of solutions and colloidal suspensions were measured by using 9,10-diphenylanthracene as a reference ($\Phi = 0.95$), while the absolute fluorescence quantum yield of DCSB ONPs was measured by using the integrating sphere equipped on FluoroMax-4-NIR spectrophotometer.

For measuring the temporally and spectrally resolved fluorescence spectra, the second harmonic (400 nm, 120 fs, 1 kHz) of a regenerative amplifier (Spitfire, Spectra Physics) seeded with a mode-locked Ti:sapphire laser (Tsunami, Spectra Physics) was used to excite the samples (liquid sample in a 10 mm cuvette or crystal sample mounted on a quartz plate) at the front surface with an incidence angle of 45°. Fluorescence collected along the sample surface normal direction was dispersed by a polychromator (250is, Chromex) and detected with a streak camera (C5680, Hamamatsu Photonics). The spectral resolution was 0.2 nm, and the temporal resolution was about 2 ps. For two-photon pumped single-crystal lasing experiment, the excitation light directly came from the fundamental (800 nm, 120 fs, 1 kHz) of the regenerative amplifier (Spitfire, Spectra Physics) seeded with a mode-locked Ti:sapphire laser (Tsunami, Spectra Physics) with the excitation fluence adjusted by using a set of neutral density filters.

TPA cross sections were determined via a comparative method by measuring the TPEF using Rhodamine B as a reference. The fundamental of a mode-locked Ti:sapphire laser (690-850 nm, Tsunami) was focused into a quartz cuvette having an optical geometry and detected with a liquid-nitrogen-cooled charge-coupled device (CCD) (SPEC-10-400B/LbN, Roper Scientific) attached to a polychromator (Spectropro-550i, Acton).

6. Cytotoxicity of the DCSB nanoprobes

To assess the cytotoxicity of the DCSB nanoprobes, NIH-3T3, A549 cells (human epithelial lung cancer) were grown in the presence of DCSB nanoparticle, and the viability was measured using a CCK-8 assay. Cells were cultured in a 96-well microplate (approximately 2000 cells per well) with a medium containing various concentrations of DCSB nanoparticles for 24 h at 37 °C in a humid atmosphere of 95% air and 5% CO₂. After the culture medium was removed, 100 µL of fresh culture medium (DMEM) containing 10% CCK-8 reagent was added to each well and the cells were incubated at 37 °C for an additional 2 h. The quantification of cell viability was determined using an ELISA microplate reader at an optical absorbance of 450 nm. The cell viability was calculated as the ratio of the absorbance of the sample well to that of the control well and expressed as a percentage.

7. Cell imaging

For cell imaging, the human cervix adenocarcinoma cell line HeLa and NIH-3T3 cells were cultured in 25 cm² flasks (Corning) in DMEM with 10% FBS at 37°C under 5% CO₂. After several days, the cells were put on quartzbottom dishes, and incubated for 24 h. Next, 20 µL of sterile filtered COPV nanoparticle dispersions (~ 5 mg·mL⁻¹) was added to the Cells and allowed to incubate for 12 h. The unbound nanoparticles were washed away with PBS and cell samples are fixed by 4% paraformaldehyde solution. After that, the cells were imaged in bright field and under NIR excitation using an fluorescence microscope (Olympus FV1000MAITAI) equipped with NIR laser.

8. Additional data and results

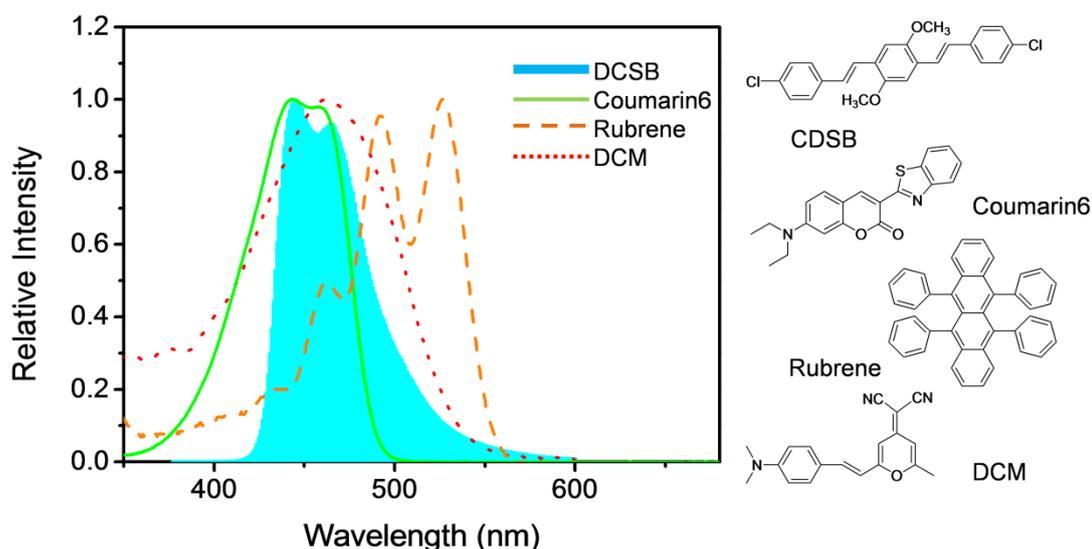


Fig. S1 Normalized fluorescence emission spectrum of DCSB nanoparticles and absorption spectra of the fluorescent dyes.

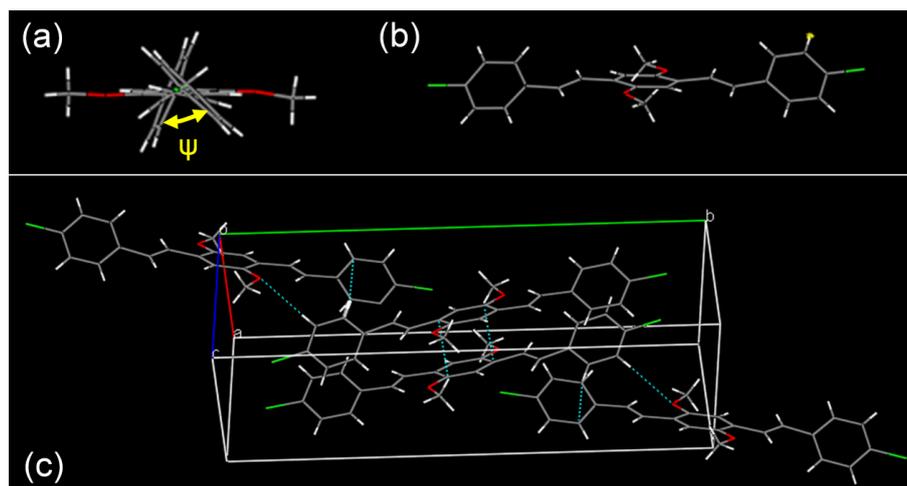


Fig. S2 (a) The X-ray single structure of the DCSB molecule from different viewpoints. The molecules exist in all-trans form in the solid state with the dihedral angle of the two adjacent benzenes of 43.78° and 69.51° , respectively. (b) Unit cell structure of DCSB crystal. Monoclinic DCSB crystal (DCSB.cif) belongs to the space group of $P 2_1/n$, with cell parameters of $a = 9.6685(19)$, $b = 22.865(5)$ Å, $c = 9.6903(19)$ Å, $\alpha = \gamma = 90^\circ$, $\beta = 112.41(3)^\circ$, $Z = 4$ and $Z' = 0$. Because of the severe distortion of the DCSB molecule, the adjacent molecules slide completely out from under one another and there is little remaining π -overlap between them.

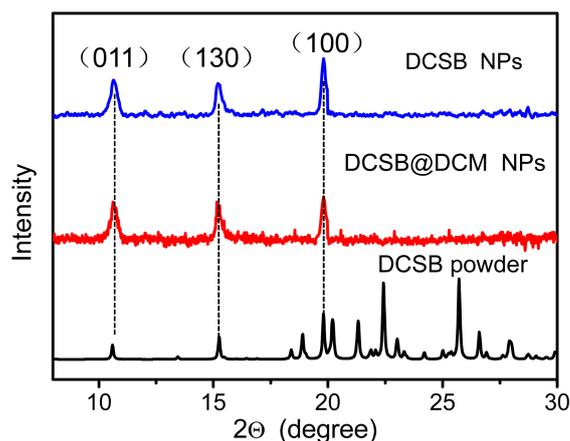


Fig. S3 XRD curves of DCSB NPs (top panel) and DCSB @ DCM NPs (middle panel) filtered on the surface of an AAO membrane with a pore size of 20 nm. For comparison, simulated powder spectra (bottom panel) are also included.

1. It can be seen that all the XRD peaks detectable in the spectrum of nanoparticles (top) can be perfectly indexed to the crystal structure (bottom), but are broad in common.
2. We calculated the crystal dimension according to Scherrer equation, $\chi = K\lambda/\beta\cos\theta$, where χ is the mean of crystal dimension, K is the shape factor which has a typical value of about 0.9, λ is the X-ray wavelength of 1.54 Å, β is the line broadening at half the maximum intensity in radians, and θ is the Bragg angle. The result shows that the average grain size is about 4-7 nm. As nanoparticles of DCSB have an average size of 40 nm, we considered that they are polycrystalline in nature lack of long-range order.
3. X-ray diffraction (XRD) results of dye-doped ONPs filtered on the surface of alumina membrane reveal weakly resolved peaks from DCSB matrix but no signals from dopant dyes, suggesting that dopant dyes are embedded in DCSB polycrystalline

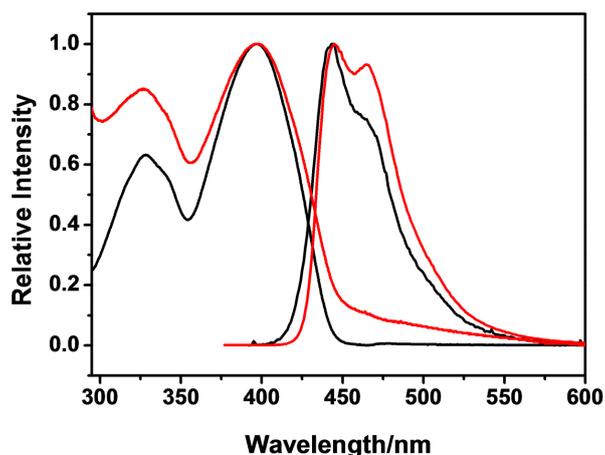


Fig. S4 Steady-state absorption (solid) and fluorescence (dash) spectra of DCSB molecules in the THF solution (black) and ONPs dispersed in THF:water =1:9 (v:v) mixture (red) with a concentration of 1.0×10^{-5} M.

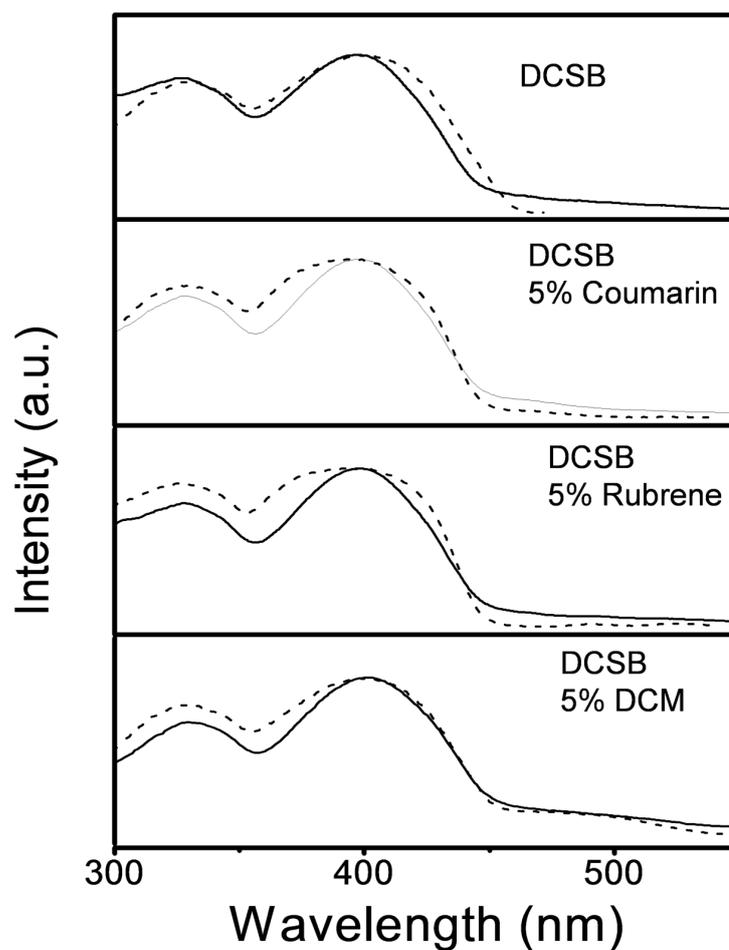


Fig. S5 Normalized absorption (solid lines), fluorescence excitation (dashed lines) of pure and dye-doped DCSB nanoparticles. The excitation spectra of the dispersion monitored at the dopant dyes emissions agree well with their absorption spectra, revealing energy levels including not only the dopant but also DCSB molecules. That's another clear evidence for the efficient energy transfer from the donor DCSB to the acceptor.

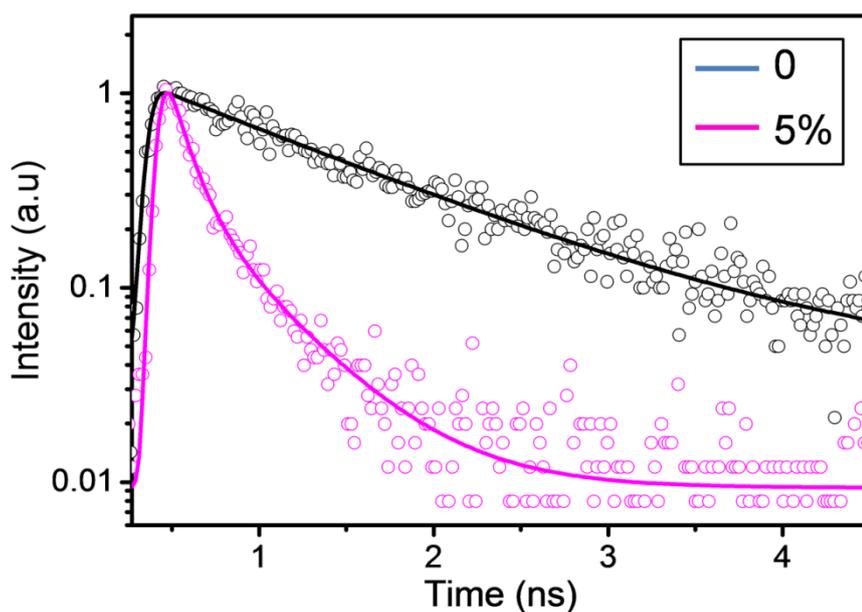
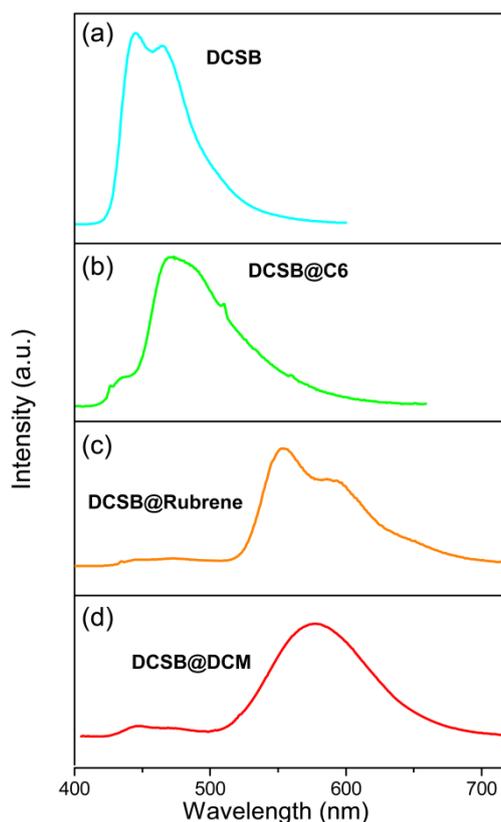


Fig. S6 Fluorescence decay curve of suspensions of the pure and DCM-doped DCSB NPs.

The efficiency of the FRET process in the doped nanoparticles can be obtained from the comparison of the fluorescence decay curves of the donor in the pure and doped NPs according to the equation:

$$\phi_{FRET} = 1 - \frac{\int_0^{\infty} I_f^{D+A}(t) dt}{\int_0^{\infty} I_f^D(t) dt}$$

In the photophysical characterization of the pure and doped NPs, $I_f^D(t)$, $I_f^{D+A}(t)$, have been obtained. We can measure the FRET quantum yield $\phi_{FRET} = 0.76$.



ONPs	C^a	Φ	η^b
DCSB	/	0.75	/
@5%C6	0.20	0.73	0.80
@5%Rubrene	0.18	0.74	0.82
@5%DCM	0.23	0.58	0.82

^aThe contribution of the DCSB emission to the emission of the doped ONPs. ^bET efficiency from donor DCSB to acceptor dopant dyes.

Fig. S7 TPEF (colored dashed lines) spectra of pure DCSB (a) and dye-doped ONPs containing 5% of C6 (b), rubrene (c) and DCM (d).

The efficiency of the energy transfer (ET) process in the doped nanoparticles can be obtained from the contribution of the DCSB emission to the emission of the doped ONPs and the quantum yield of the DCSB undoped and doped ONPs according to the equation:

$$\eta = 1 - C * \Phi_{DOPEd} / \Phi_{DCSB}$$

In the photophysical characterization of the pure and doped NPs, Φ_{DCSB} , Φ_{DOPEd} have been obtained. We can measure the ET quantum yield.

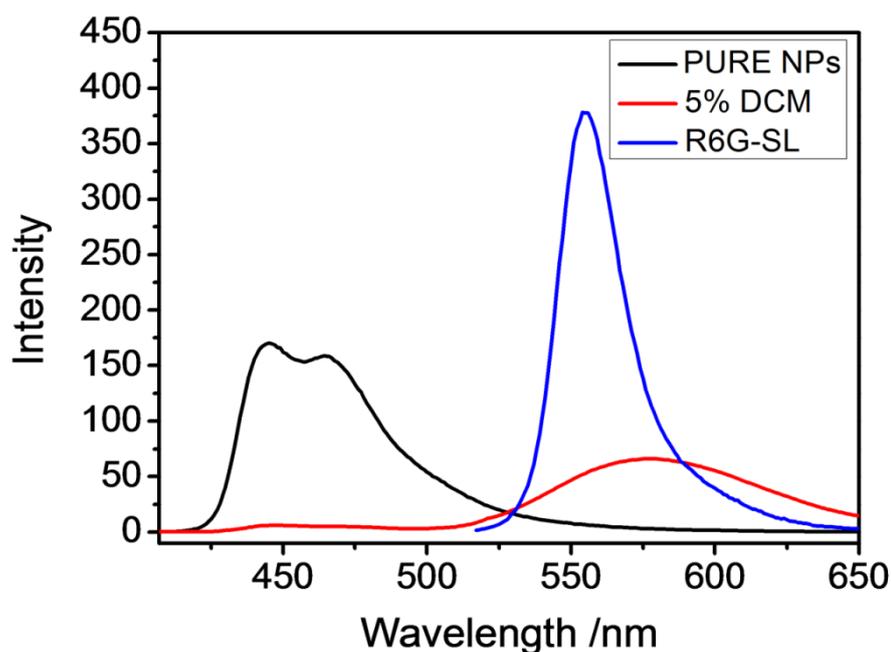


Fig. S8 Steady-state fluorescence spectra of pure DCSB ONPs (black) and DCSB@5% DCM ONPs (red) dispersed in THF: water = 1:9 (v:v) mixture and R6G molecules in the ethanol solution (blue) with a concentration of 1.0×10^{-5} M.

1. For comparison, all the spectra were measured with the same spectrometer. The excitation wavelength was 397 nm (pure NPs and DCM doped NPs) and 507 nm (R6G monomers), respectively, in order to ensure the emission spectra were measured at the same absorbance intensity (the intensity of the absorbance $A=0.45$).
2. As the average diameter of the COPV NPs is 40 nm, and the cell (4 molecules) volume of DCSB is 1.98045 nm^3 , there are 8.899×10^{13} NPs in the measured suspension with a concentration of 1.0×10^{-5} M.
3. We calculated the brightness according to the equation:

$$NP_{\text{relative brightness}} = \frac{FL_{NP} / C_{NP}}{FL_R / C_R}$$

where FL_{NP}/C_{NP} is the (integral) amount of fluorescent light coming from DCSB NPs, and C_{NP} (C_R) is the density of DCSB NPs (dye concentration) in the measured suspension (solution). The integral fluorescence of pure DCSB NPs, doped NPs and R6G monomers was 10033 (a.u.), 6337 (a.u.) and 12542 (a.u.) respectively, so the fluorescent brightness of the single pure or doped NPs is equivalent to the brightness of approximately 5.4×10^4 molecules or 3.4×10^4 of the monomer R6G. This is reported that a single ZnS-capped CdSe quantum dot is ≈ 20 times brighter than a molecule of R6G. Thus the brightness of pure DCSB NPs or doped NPs is 2.7×10^3 times or 1.7×10^3 higher than a single ZnS-capped CdSe quantum dot.

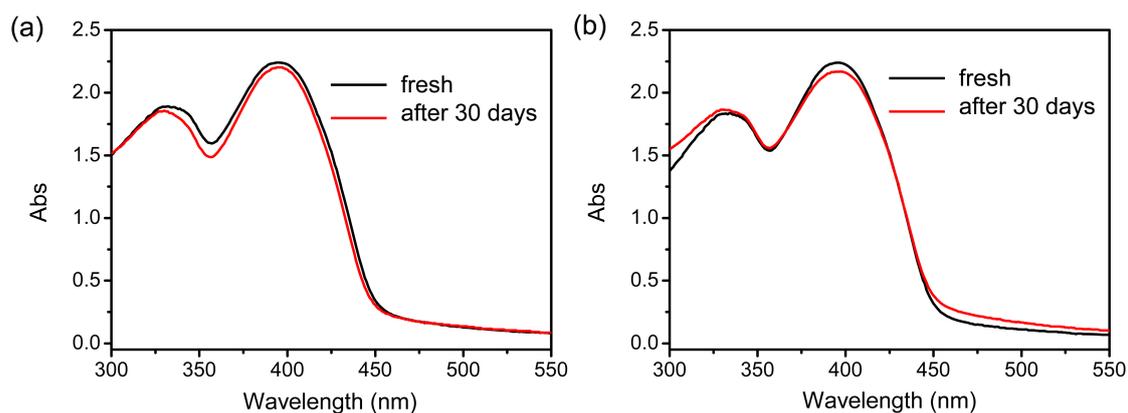


Fig. S9 Absorption spectra of ONPs dispersed in water (a) and PBS (b) solution for 30 days. As shown in fig.S9, the absorption spectra of the ONPs dispersed in water and PBS solution at high concentration ($5 \times 10^{-5} \text{ mol} \cdot \text{L}^{-1}$) show no obvious change for 30 days. The results suggest that the aqueous and PBS suspensions keep stable at ambient conditions and without any noticeable aggregation even at extremely high concentration for a long time.

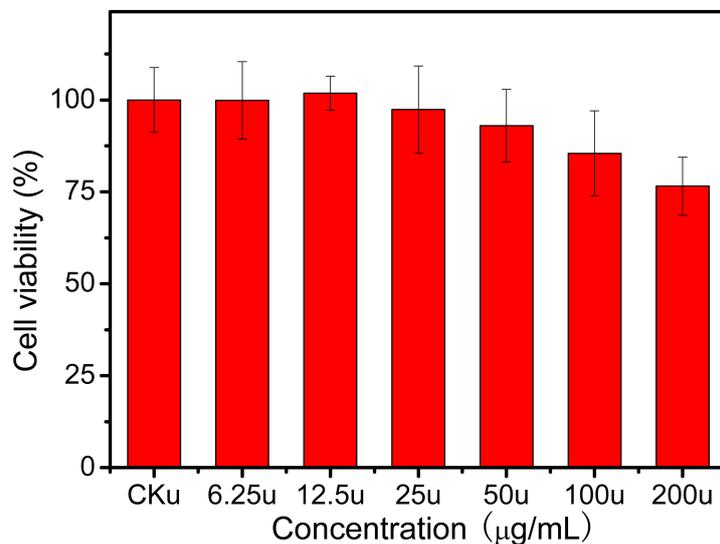


Fig. S10 Viability of A549 cells at various concentrations of DCSB@5%DCM ONPs.

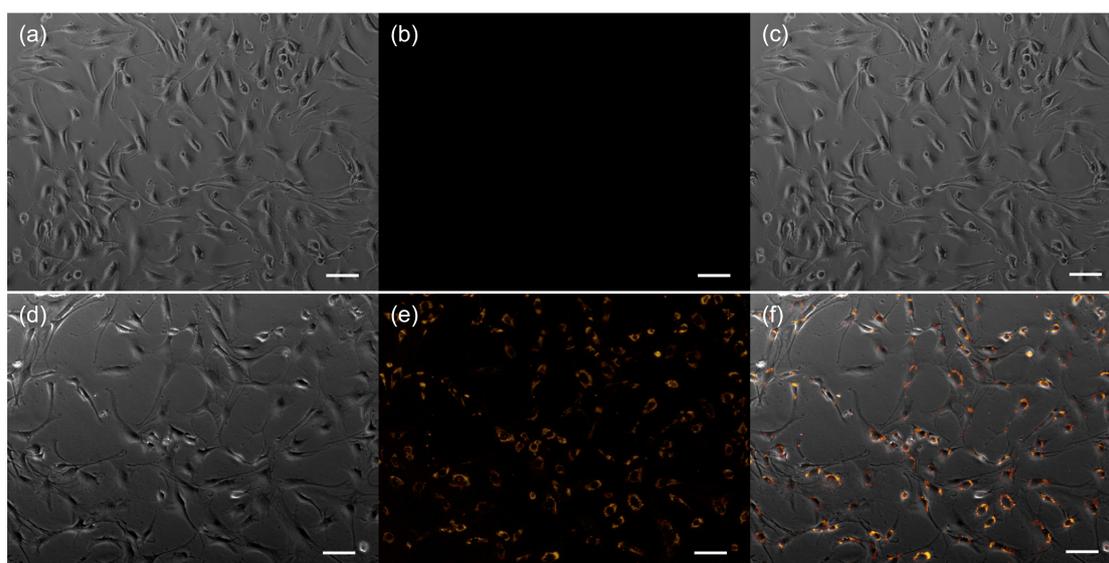


Fig. S11 One-photon-excitation fluorescence images of NIH-3T3 cells (b) without and (e) with the incubation of DCSB@5%DCM ONPs. The images on the left (a&d) are the corresponding bright-field images. The images on the right (c&f) are the overlaid pictures of the fluorescence images and the corresponding Bright-field images. The scale bar is 40 μm .

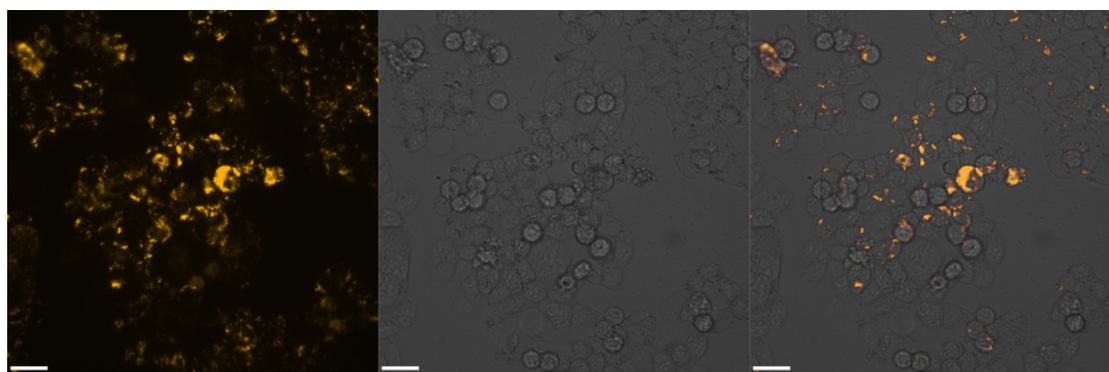


Fig. S12 One-photon excitation fluorescence images of HeLa cells with the incubation of DCSB@5%DCM ONPs. The images on the middle are the corresponding Bright-field images. The image on the right is the overlaid pictures of the fluorescence images and the corresponding Bright-field images. The scale bar is 40 μm .