

Supporting Information

Water-soluble J-aggregate Nanoparticle as an Efficient Two-Photon Fluorescent Nano-Probe for Bio-Imaging

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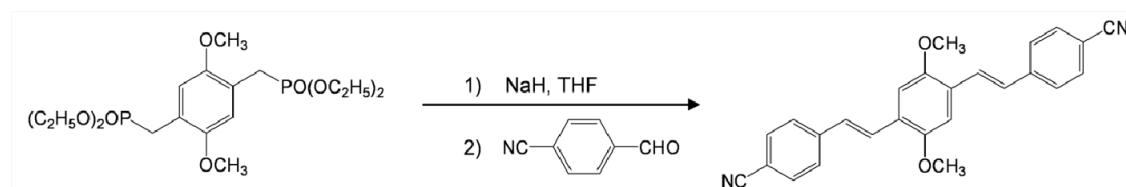
Experimental

1. Materials

The compound used in our work, cyano-terminated OPV (COPV) was synthesized as described below. All starting materials were purchased from Sigma-Aldrich and used as received without further purification. The solvent of 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, 0.1 μm) were bought from Whatman International Ltd.

2. Synthesis of COPV

General Procedure :



To the 2,5-bis(methoxy)-1,4-xylene-bis(diethyl phosphonate) (1.00 g, 2.25 mM) and the 4-cyanobenzaldehyde (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 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 by means of CH_2Cl_2 /petroleum ether (1:4). And COPV 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 ; $^1\text{H NMR}$ (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, $-\text{OCH}_3$); MS (EI): 392

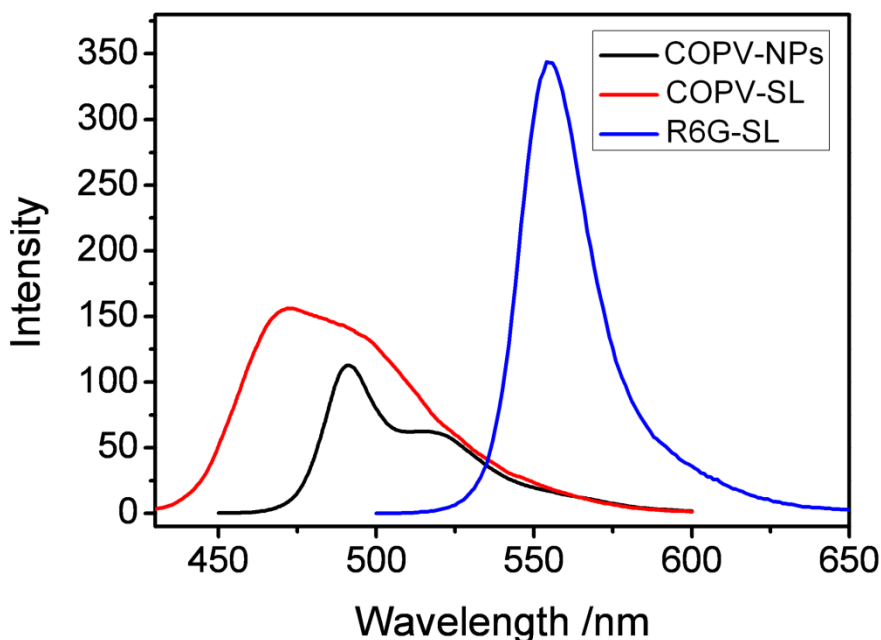


Fig. S1 Steady-state fluorescence spectra of COPV NPs dispersed in THF: water = 1:9 (v:v) mixture (black) and COPV molecules in the THF solution (red) and R6G molecules in the ethanol solution (black) with a concentration of 1.0×10^{-5} M.

1. For comparison, all the spectra were measured with the same spectrometer. The excitation wavelength was 344 nm (COPV NPs and COPV monomers) and 494 nm (R6G monomers), respectively, in order to ensure the emission spectra were measured at the same absorption intensity ($A=0.32$).
2. As the average diameter of the COPV NPs is 95 nm, and the cell (2 molecules) volume of COPV is 1.04523 nm^3 , there are 7.008×10^{12} NPs in the measured suspension with a concentration of 1.0×10^{-5} M.
3. We calculated the brightness according to the equation:

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

where FL_{NP}/C_{NP} is the (integral) amount of fluorescent light coming from COPV NPs, and C_{NP} (C_R) is the density of COPV NPs (dye concentration) in the measured suspension (solution). The integral fluorescence of COPV NPs, COPV monomers and R6G monomers was 4888 (a.u.), 10143 (a.u.) and 11341 (a.u.) respectively, so the fluorescent brightness of the single NPs is equivalent to the brightness of approximately 4.3×10^5 molecules of the monomer COPV or 3.7×10^5 molecules 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 COPV NPs is 1.8×10^4 times higher than a single ZnS-capped CdSe quantum dot.

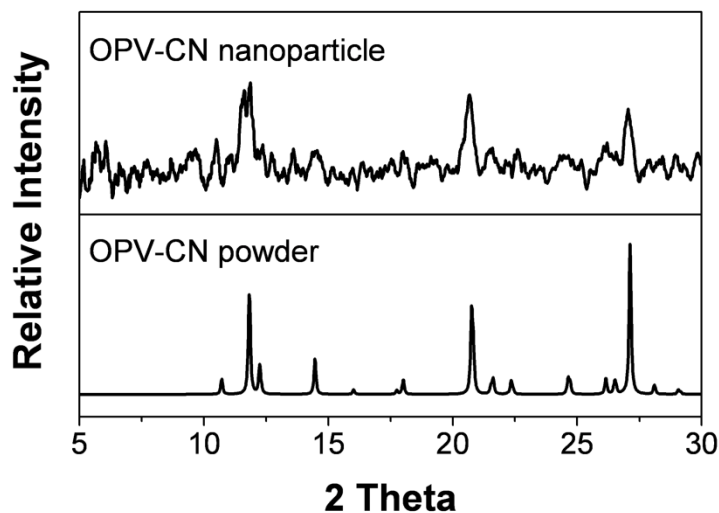


Fig. S2 XRD curves of nanoparticles (top panel) of COPV filtered on the surface of an AAO membrane with a pore size of 20 nm. For comparison, simulated powder spectra (bottom panel) are also included.

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 5-10 nm. As nanoparticles of COPV have an average size of 95 nm, we considered that they are polycrystalline in nature lack of long-range order.

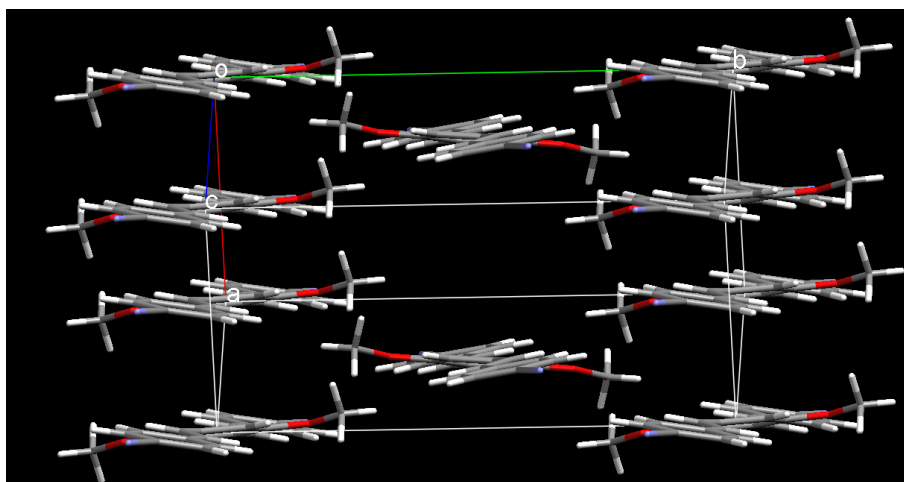


Fig. S3 Unit cell structure of COPV crystal. Monoclinic COPV crystal (COPV.cif) belongs to the space group of $P 2_1/c$, with cell parameters of $a = 8.5110(15) \text{ \AA}$, $b = 14.954(3) \text{ \AA}$, $c = 8.4748(15) \text{ \AA}$, $\alpha = \gamma = 90^\circ$, $\beta = 104.292(12)^\circ$, $Z = 2$ and $Z' = 0$. Within ac plane, COPV molecules form π -stacked two-dimensional layer structures assisted by hydrogen-bonding interactions. As shown in Figure 2c in the manuscript, brickwork arrangement of COPV molecules results in the formation of J-aggregates. Moreover, it can be seen that those 2D π -stacked layers pack along the crystal b -axis via interdigitation of methoxyl groups.

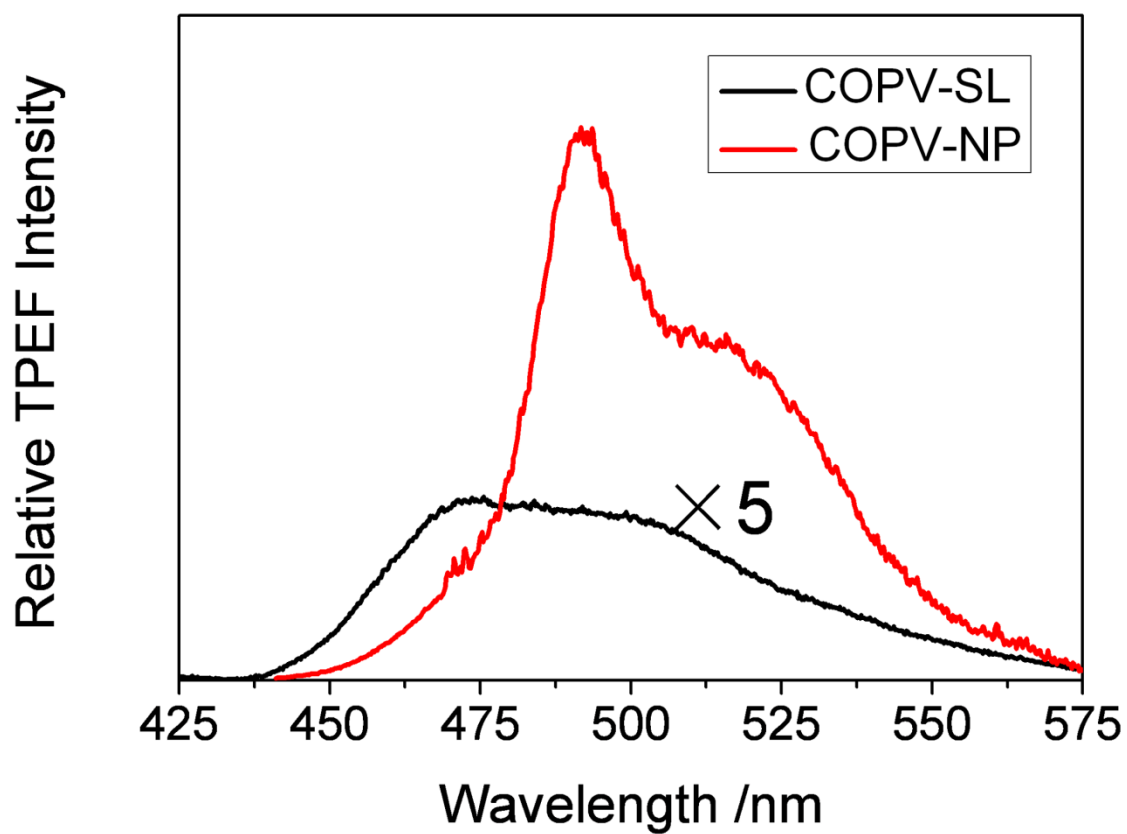


Fig. S4 Spectra for COPV solution in THF (black) and nanoparticles dispersed in THF: water = 1:9 (v:v) mixture (red), excited at 800 nm.

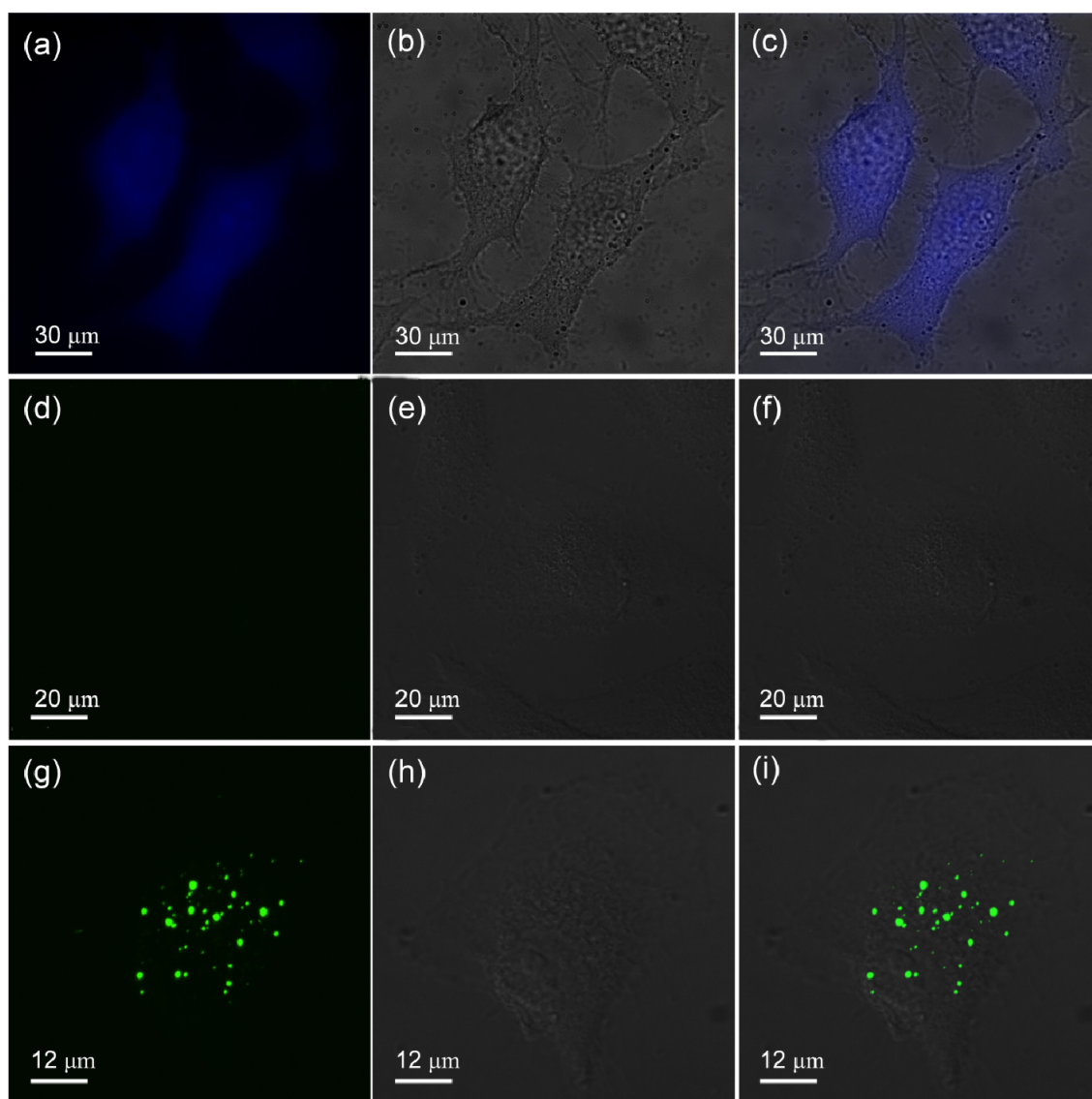


Fig. S5 One-photon (a) and two-photon (c) excitation fluorescence images of HeLa cells without the incubated with COPV NPs. The images on the middle (b&e) are the corresponding Bright-field images. The images on the right (c&f) are the overlaid pictures of the fluorescence images and the corresponding Bright-field images. (g)TPEF image of HeLa cells that were incubated with NPs of COPV upon excitation at 830 nm. (h) The bright-field images of HeLa cells that were incubated with J-aggregate NPs of COPV (i) The overlaid picture of TPEF and corresponding bright-field images.

It can be seen that the strong background fluorescence from HeLa cells was excited at the high one-photon excitation (408nm) energy. Intense intracellular luminescence was observed under excitation of femtosecond laser pulses at 830 nm, which factually do not excite any background fluorescence from HeLa cells.

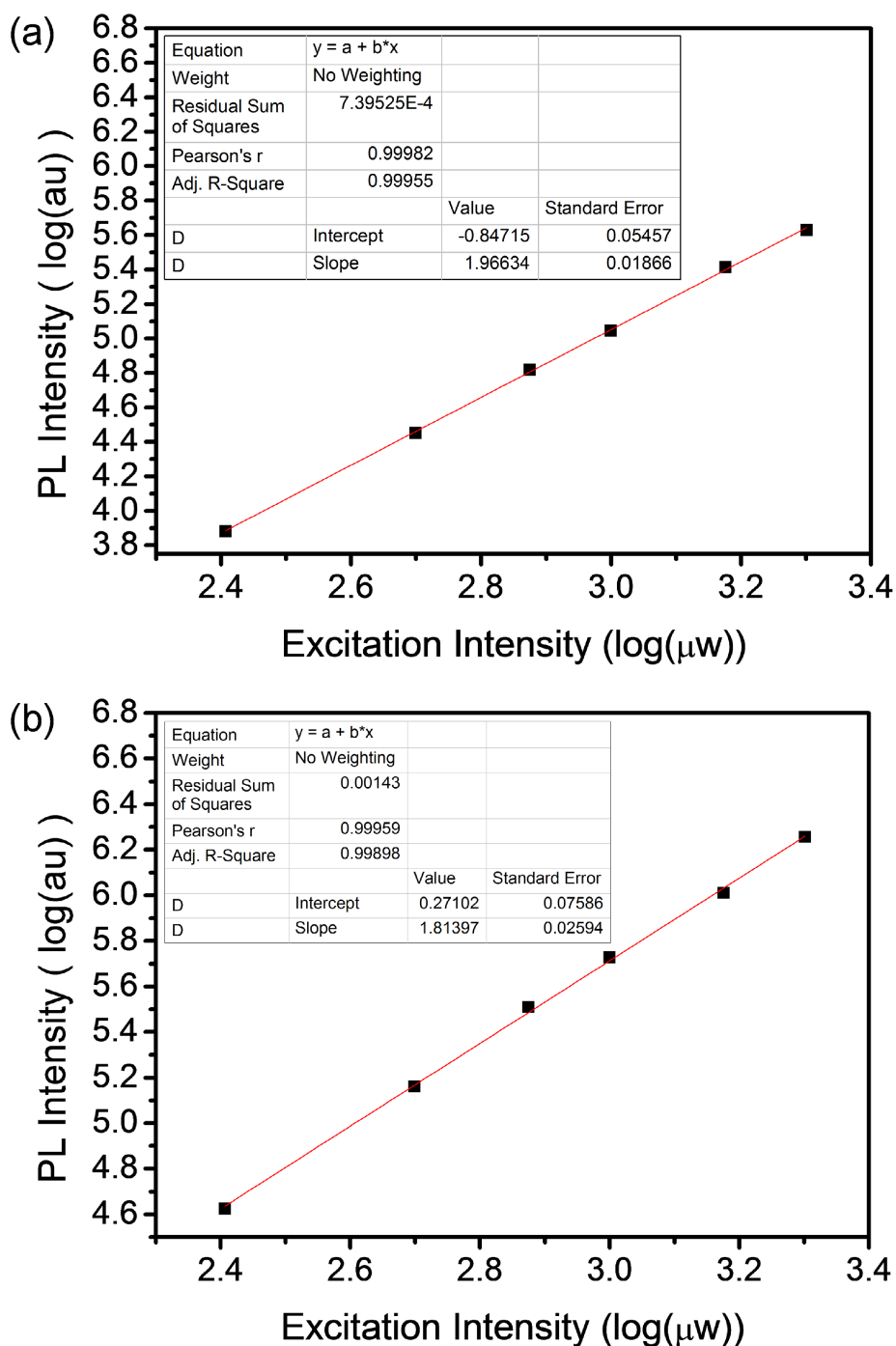


Fig. S6 The peak intensity of two-photon excited fluorescence spectra versus laser intensity at 800 nm for COPV solution in THF (a) and nanoparticles dispersed in THF: water = 1:9 (v:v) mixture(b). The log–log plots show slopes of 1.97, 1.81 which validate two photon nature of the absorptions. The excitation was 800 nm and the emission were 474 nm and 491 nm, respectively.