Supporting Information

Functionalized AIE nanoparticles with efficient deep-red emission, mitochondria specificity, cancer cell selectivity and multiphoton susceptibility

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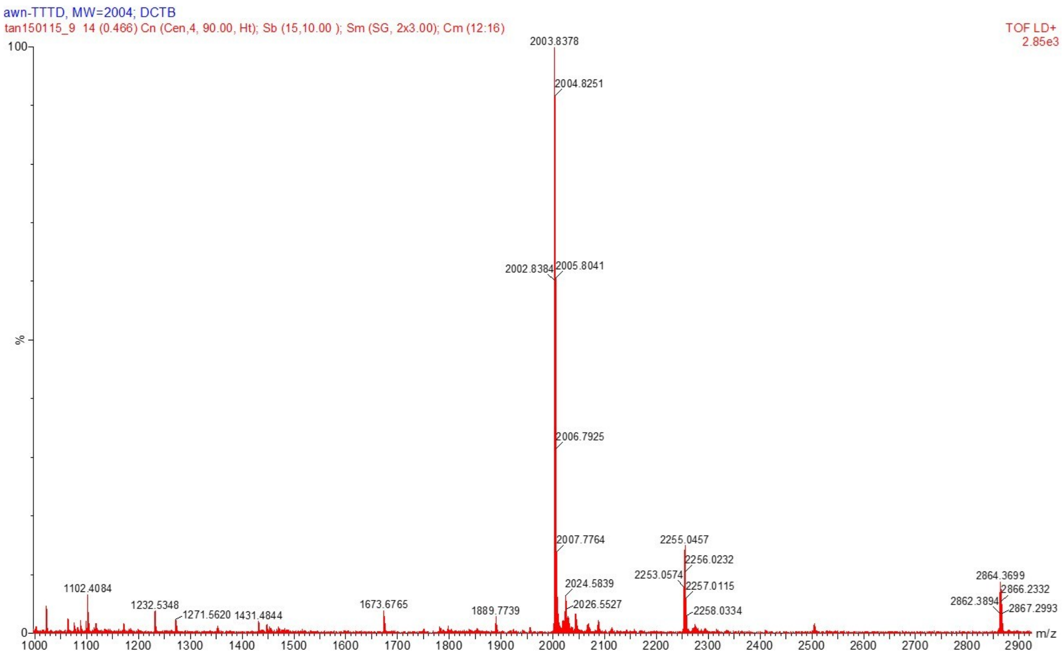


Figure S1. HRMS spectrum of TPE-TETRAD.

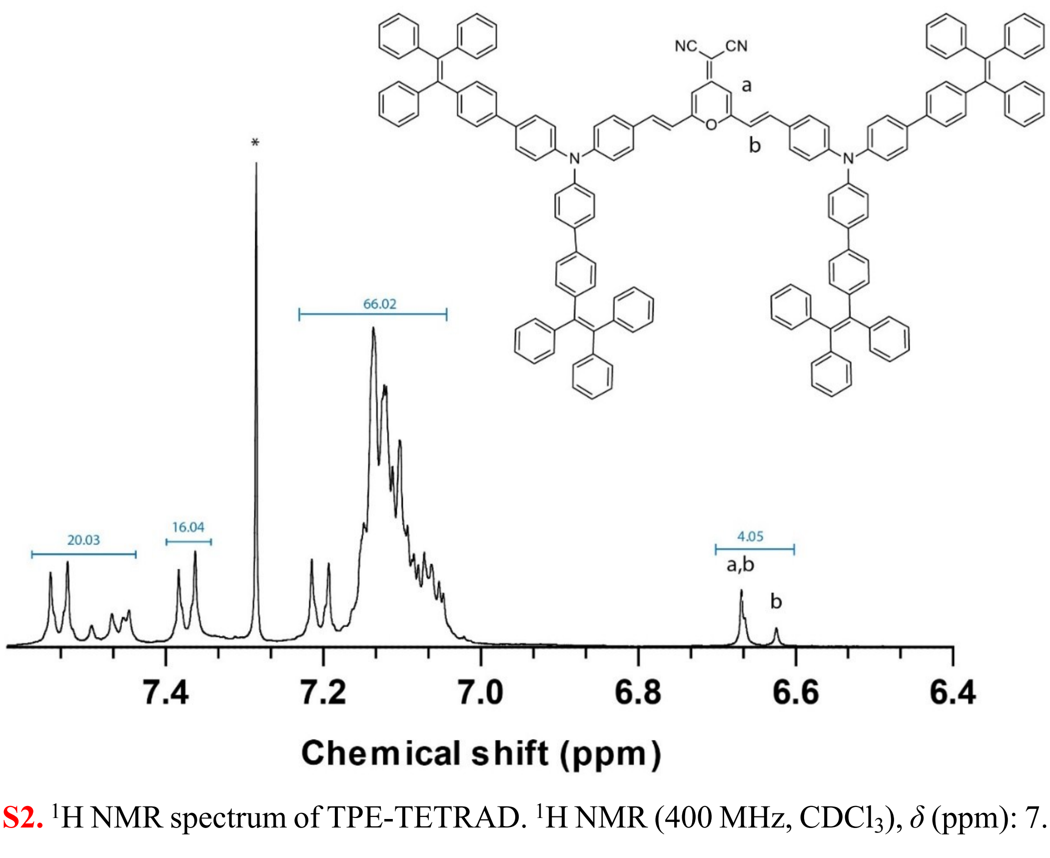


Figure S2. ^{1}H NMR spectrum of TPE-TETRAD. ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 7.56–7.43 (m, 20H), 7.39–7.34 (m, 16H), 7.33–7.0 (m, 66H), 6.67 (d, J = 16 Hz, 2H; pyran –CH=), 6.63 (s, 2H; pyran H).

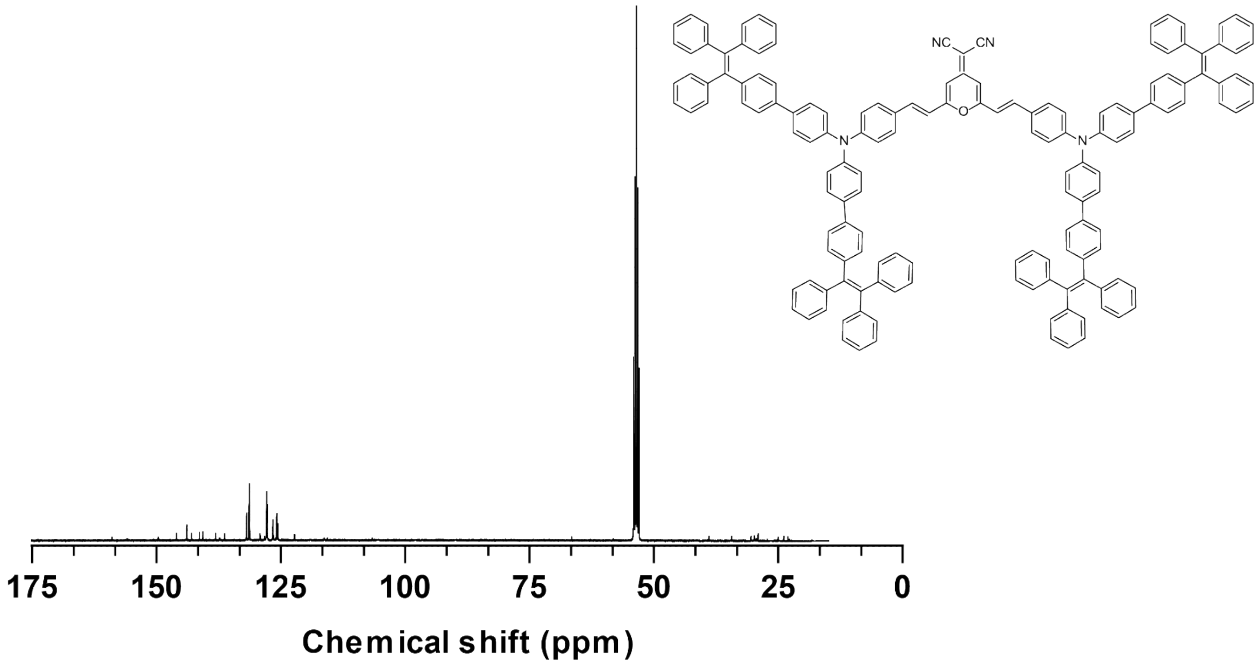


Figure S3. ^{13}C NMR spectrum of TPE-TETRAD. ^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 159.5, 150.0, 145.9, 143.8, 142.7, 141.2, 140.5, 139.1, 136.2, 131.6, 131.2, 128.7, 127.7, 126.2, 125.7, 122.1, 116.5, 115.2, 106.4, 53.4.

Table S1. Optical Properties of TPA-DCM and TPE-TETRAD

	λ_{ab} (nm)[a]	E_g (eV)[b]	λ_{em} (nm)[c]		Stokes shift[d]
			solution	aggregate	
TPA-DCM	465	2.72	620	670	155
TPE-TETRAD	500	2.56	668	675	168

[a)]Absorption maxima (λ_{ab}) in THF(10 μM); [b)]HOMO-LUMO band gap (E_g) derived from theoretical DFT calculations; [c)]emission maxima (λ_{em}) for solution (in THF,10 μM) and aggregate (in THF/water 1:9 v/v, 10 μM); [d)]Stokes shift derived from subtracting λ_{em} from λ_{ab}.

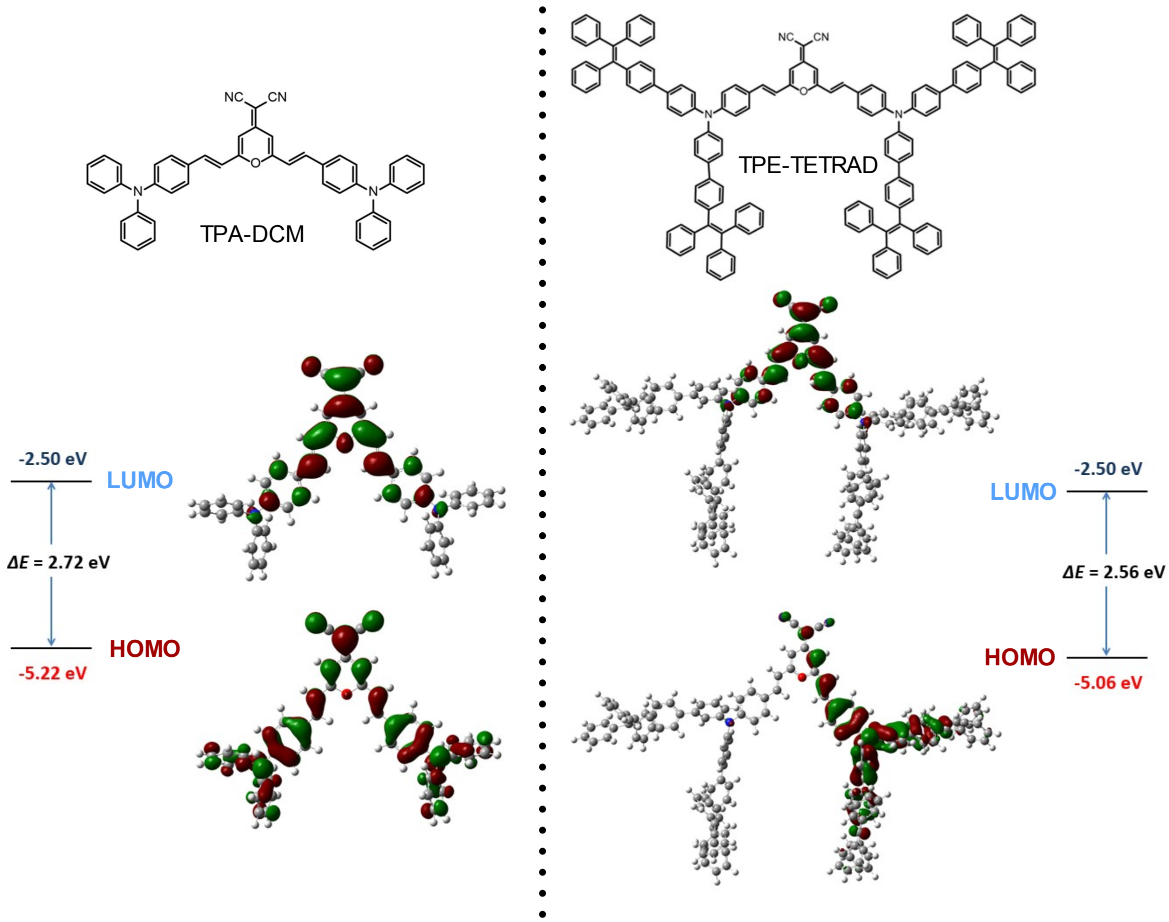
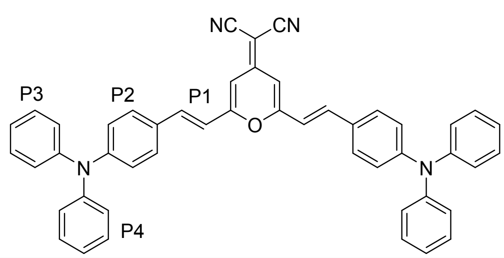
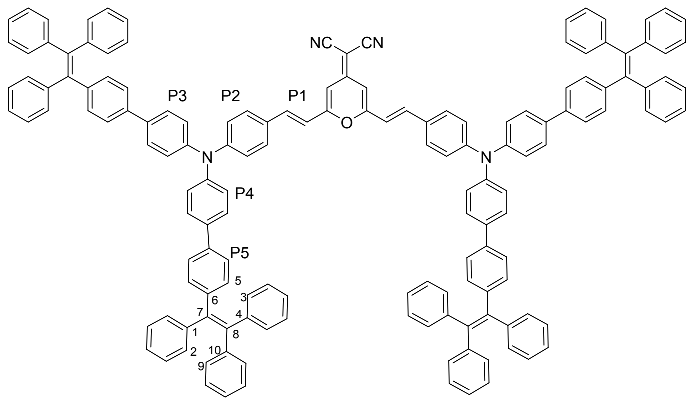


Figure S4. HOMO and LUMO energy levels of TPA-DCM and TPE-TETRAD, respectively. Molecular orbital amplitude plots of HOMO and LUMO energy levels of TPA-DCM and TPE-TETRAD were calculated using density functional theory (DFT) in Gaussian 09 program using B3LYP/6-31G(d) basis set.



Angles between planes	
P_1-P_2	~0
P_2-P_3	~67
P_2-P_4	~67
P_3-P_4	~76

Figure S5. Summary of dihedral angles (°) for TPA-DCM. The dihedral angles between any two phenyl rings of TPA in TPA-DCM are from ~67° to ~76°.



Dihedral angles (°)	
C5-C6-C7-C8	-48.4
C3-C4-C7-C8	-49.9
C2-C1-C7-C8	-50.0
C9-C10-C8-C7	-49.7
Angles between planes	
P_1-P_2	~0
P_2-P_3	~68
P_2-P_4	~67
P_3-P_4	~75
P_4-P_5	~35

Figure S6. Summary of dihedral angles (°) for TPE-TETRAD. In the TPE component of TPE-TETRAD, the dihedral angles between any phenyl rings are ~50°. The dihedral angles between any two phenyl rings of TPA in TPE-TERAD are from ~67° to ~75°.

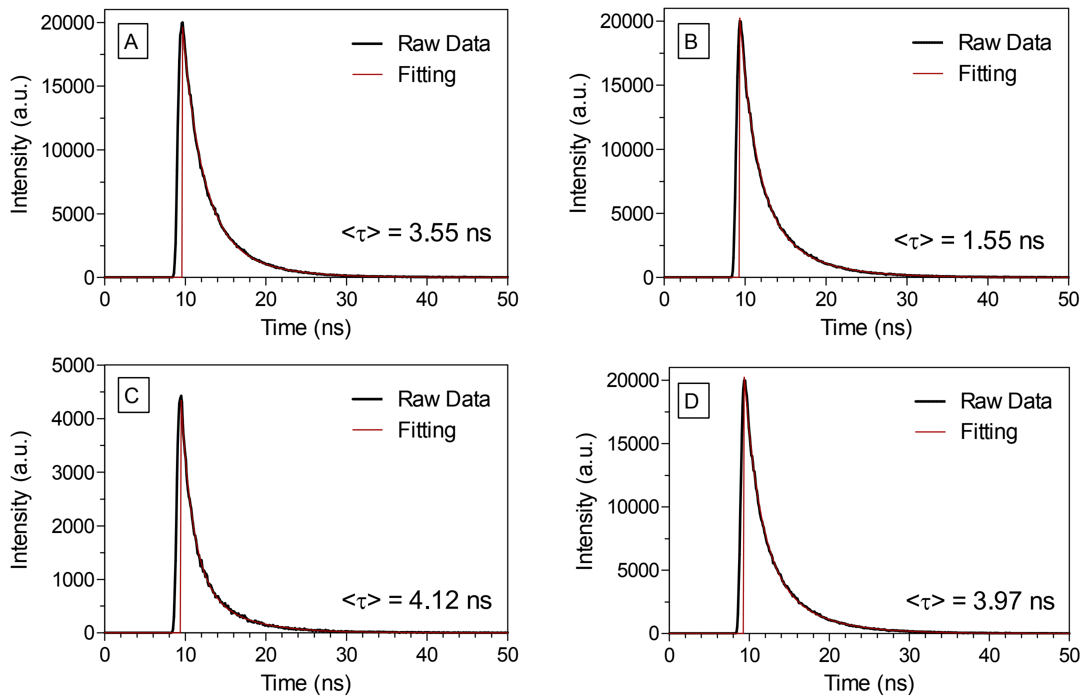


Figure S7. Fluorescence lifetime of (A) TPE-TETRAD thin film solid, (B) TPE-TETRAD nanoaggregates (10 μM) in water, (C) TPE-TETRAD@Biotin NPs (6 μg/mL) in water and (D) TPE-TETRAD (10 μM) in THF.

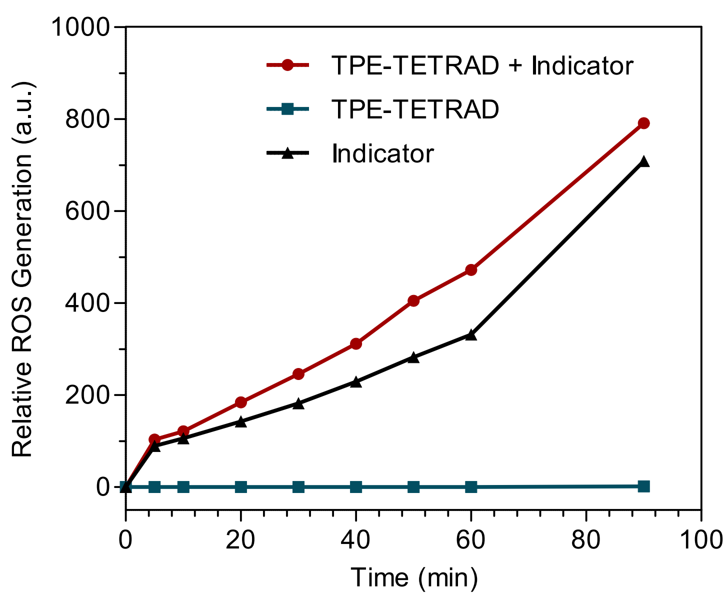
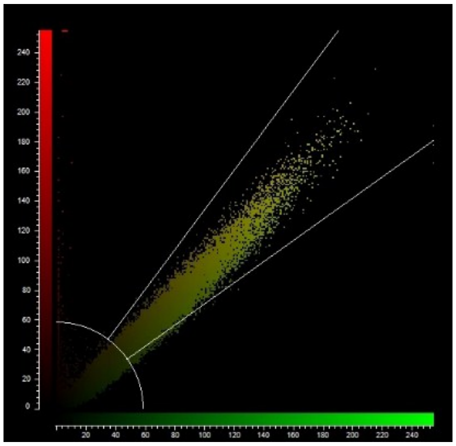


Figure S8. Relative ROS generation profile for TPE-TETRAD using DCFH-DA cellular reactive oxygen species detection assay kit. Change in fluorescent intensity at 534 nm for TPE-TETRAD, DCFH-DA indicator and their mixture in PBS upon white light irradiation for various time intervals. Excitation wavelength: 485 nm. [TPE-TETRAD] = 10 μM; [DCFH-DA] = 1 μM.



Colocalization Statistics

Pearson's Correlation	0.919
Overlap Coefficient	0.9288
Colocalization Rate	94.53%
Colocalization Area	306.53 ÂµmÂ²
Area Image	5302.86 ÂµmÂ²
Area Foreground	324.27 ÂµmÂ²
Area Background	4978.70 ÂµmÂ²

Figure S9. CLSM colocalization statistics for HeLa cervical cancer cells stained with 25 nM MitoTracker Green for 10 min and 6 μg/mL TPE-TETRAD@Biotin NPs for 1 h at 37 °C.

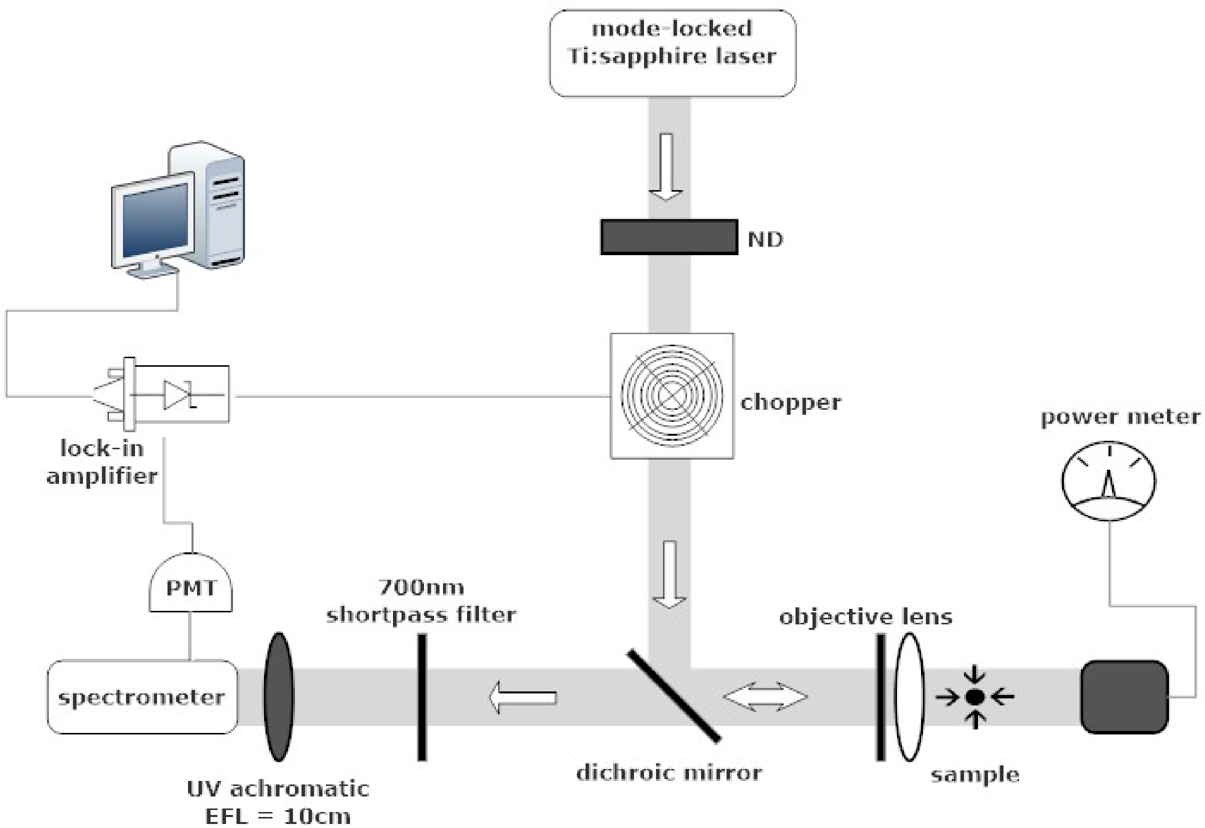


Figure S10. Schematic illustration of the experimental setup for two-photon excited fluorescence.

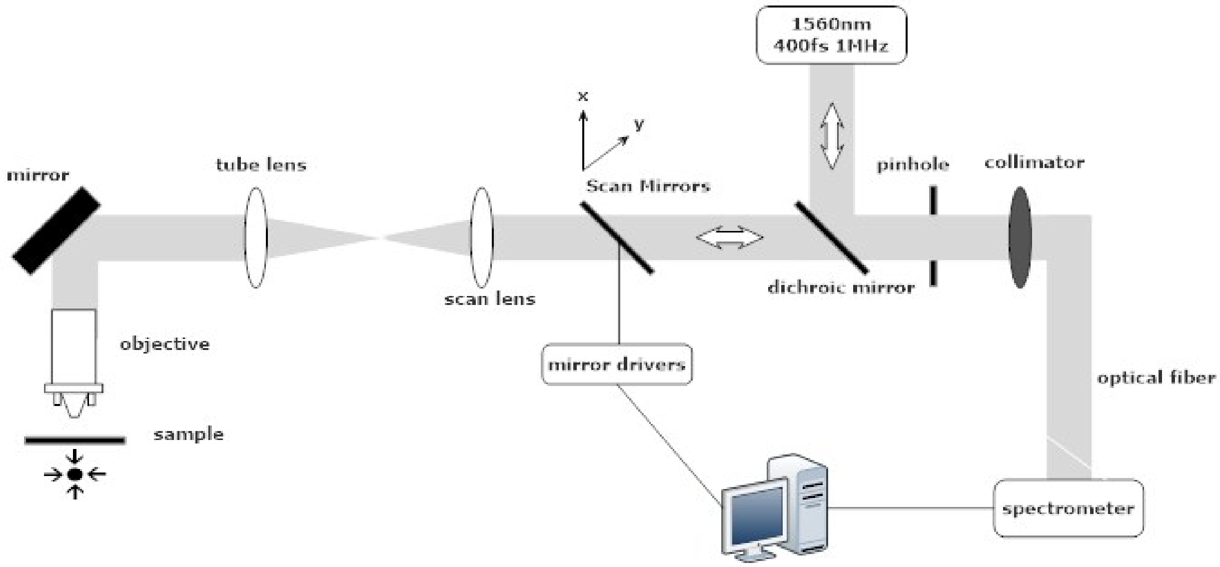


Figure S11. Schematic illustration of the NLO microscope system for spectrum measurement.

Calculation of TPE-TETRAD@Biotin AIE NPs Concentration

The density of TPE-TETRAD@Biotin can be estimated as 1 g/cm^3 due to its high water stability. The average size of the AIE NPs as verified by TEM and DLS was 100 nm. 0.25 mg of powder was obtained after freeze-drying 1 mL of the filtered stock solution. The TPE-TETRAD@Biotin concentration in a 1 mL stock suspension can be calculated from the following equation:

$$\frac{Total\ volume\ of\ TPE-TETRAD@Biotin\ NP}{Average\ volume\ of\ each\ NP} = \frac{\frac{0.25\ x\ 10^{-3}}{1g/mL}}{\frac{4}{3}\pi\ x\ (50\ x\ 10^{-7})^3 mL} = 4.78\ \text{x}\ 10^{11}$$

, Total number of NP in 1mL.

$$[\text{TPE-TETRAD@Biotin}] = \frac{\frac{4.78\ x\ 10^{11}}{6.02\ x\ 10^{23}\ mol^{-1}}}{1\ x\ 10^{-3} L} = 0.8\ \text{nM}$$

, final concentration in stock solution

$$\text{Working Concentration} = \frac{0.8nM\ x\ 50\mu L}{2000\mu L} = 20\ \text{pM}$$

, final working concentration for cell imaging

Or alternatively we can calculate the mass concentration:

$$\text{Working Concentration} = \frac{250\mu g/mL\ x\ 50\mu L}{2000\mu L} = 6.25\ \mu\text{g/mL}$$

, final working concentration for cell imaging.