Electronic Supplementary Information

Two-photon excitation nanoparticles for photodynamic therapy

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Table S1 Summary of TPE NPs reported to date for PDT applications.

Author	TPE NPs	δ^a (GM)	Method	Ref.
Dayal et al.	CdSe QDs-PC 4	1000-100000	TPEF [c]	79
Wen et al.	CdTe QDs-TSPP	NA[b]	NA	80
Skripka et al.	CdSe/ZnS QDs-Ce6	3325	TPEF	81
Qi et al.	CdSe/ZnS QDs-TrisMPyP-COOH	800-2400	TPEF	82
Fowley et al.	CdSe/ZnS QDs-RB	~ 50000	TPEF	83
Chou et al.	CdSe/CdS/ZnS QDs-AlPcS	50000	TPEF	84
Lemon et al.	CdSe QDs-PdPS	NA	NA	85
Fowley et al.	Carbon dots-PTIX	NA	NA	94
Wang et al.	Carbon dots-TMPyP	15000	TPEF	95
Zhang et al.	SWCNTs-Ru(II) complexes	494 for Ru1 complex	TPEF	96
		428 for Ru2 complex	TPEF	96
		NA for SWCNTS	NA	96
Kim et al.	ORMOSIL-BDSA-HPPH	217 for BDSA	TPEF	100
		NA for ORMOSIL	NA	100
Cheng et al.	MSNs-FITC-PdTPP	NA	NA	101
Qian et al.	ORMOSIL-PpIX	NA	NA	102
Velusamy et al	pSiNP-11,12-dibutoxydibenzo[a,c]-phenazine bridged amines	~7000	TPEF	103
		~ 5800	Z-scan [d]	103
Gary-Bobo et al.	MSNs-Banana-shaped biphotonic quadrupolar chromophore	~ 8×10^6 per NP	TPEF	104
Secret et al.	pSiNP-Porph	360	TPEF	106
Zhao et al	AuNRs	22000-30000	TPEF	113
Jiang et al.	Aggregated AuNPs	~5000-6800	TPEF	114
Zhao et al.	AuNRs/SiO2-T790	~ 2 for T790	TPEF	115
		NA for AuNRs/SiO_2	NA	
Gao et al.	Gold nanocages-HB	NA	NA	116
Chen et al.	AuNRs- MS-PdTPP	NA	NA	117

Chen et al.	TPA block copolymer-porphyrin	288	TPEF	130
Shen et al.	PFEMO-TPP	2160	TPEF	131
Grimland et al.	PFPV-TPP	5×10^5	TPEF	132
Shen et al.	PFEMO-CO-520-TPP	2160	TPEF	133
Shen et al.	PFVCN-TPP-FA	12000	TPEF	56

[a] δ: TPA cross-section;

[b] NA: Not available;

[c] TPA cross-section (δ) was measured through two-photon excited fluorescence (TPEF) technique

[d] TPA cross-section (δ) was measured through Z-scan technique

Measurement of TPA cross-section:

Two-photon absorption (TPA) cross-section (δ) is an important parameter related to the properties (i.e., wavelength, intensity) of a molecule or material undergoing the TPA process when exposed to light of a given photon energy, allowing quantification and comparison of TPA capabilities of different molecules. Several different methods have been developed to measure TPA cross-sections, such as nonlinear transmission, Z-scan, two-photon excited fluorescence (TPEF), transient absorption, for-wave mixing technique, and so on. Among these methods, TPEF and Z-scan are the two main techniques for measuring TPA cross-sections of nanoparticles reported in Table S1. These methods are briefly described as following:

(1) TPEF technique:

The two-photon excited fluorescence (TPEF) technique is an indirect method that involves the measurement of the TPEF spectra and intensity in relative to a suitable reference material whose δ was known under identical measuring conditions (*J. Opt. Soc. Am. B* **1996**, *13*, 481; *J. Am. Chem. Soc.*, **2007**, *129*, 7220; *Angew. Chem. Int. Ed.* **2009**, *48*, 3244; *Advances in Optics and Photonics* **2010**, *2*, 451; *Chem. Eur. J.*, **2015**, *21*, 2214). **Fig. s1** shows the general experimental setup for the TPEF measurement: it requires the use of a pulse laser to generate high energy fs pulses (~ 100 fs) at a proper repetition rate; the laser beam is then focused on the sample cell (1 cm) by a lens with a proper focal length; The excitation beam was focused close to the wall of the cell in order to minimize the effects of re-absorption; the two-photon emission fluorescence was focused by a lens and detected in a direction perpendicular to the laser beam with a n optical spectrum analyzer.

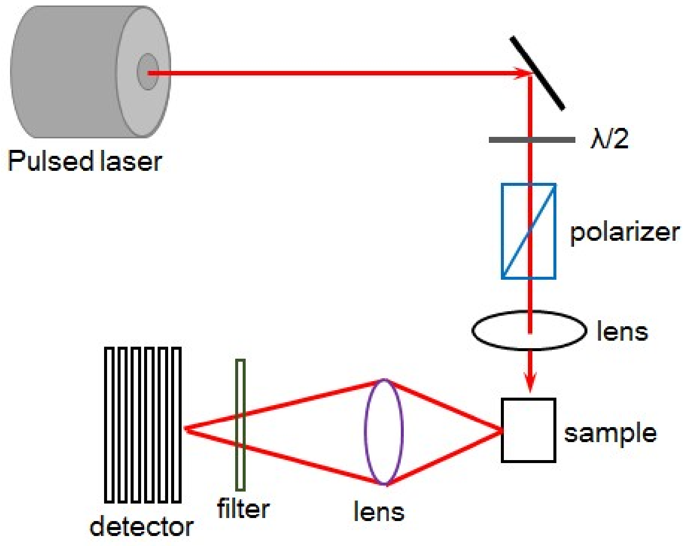

Fig. s1. Illustration of experiment set for two-photon excited fluorescence (TPEF) technique.

After acquiring the TPEF spectra of both sample and reference material under the same conditions, the δ value of the sample can be obtained using a comparison method by the following equation:

$$\delta = \delta_{ref} \frac{F \varphi_{ref} C_{ref} n_{ref}}{F_{ref} \varphi C n}$$

Where the subscript *"ref"* refers to the reference compound (For example, rhodamine 6G or rhdamine B), n is the refractive index of the solvents, φ is the fluorescence quantum yield, F is the two-photon excited integral fluorescence intensity, and C is the concentration of substrate in solution.

TPEF is a simple approach to measure the δ value of the unknown sample. As there are more and more commercially available dyes whose δ are accurately reported, the TPEF technique has become as the most popular method for the measurement of δ value. However, because the TPEF intensity increases with the square of the laser intensity, it is necessary to check the quadratic power dependence in case of overestimating the δ value. Moreover, the TPEF technique is not applicable to the samples that are non-fluorescent. In this case, other methods like Z-scan will be more suitable.

(2) Z-scan technique:

The Z-scan technique involves moving a sample along the path of a focused laser beam and measuring the light intensity at the detector as a function of its position along this z-axis (*Angew. Chem. Int. Ed.* **2009**, *48*, 3244; *J. Opt. Soc. Am. B* **2010**, *27*, 2290; *Adv. Funct. Mater.*, **2009**, *19*, 2388). **Fig. s2** shows the general experimental setup for the Z-scan: the experiment normally requires an fs laser (or ns laser) that produces a single Gaussian pulse, which is then coupled to a regenerative amplifier to generate a high energy pulse; after passing through lens (f = 20 cm), the laser beam is focused and passed through a cell (thickness = 2. 00 cm) filled with the sample solution; when the sample cell changes its position along the beam direction (z-axis), the transmitted laser beam from the sample cell is detected by a photon detector.

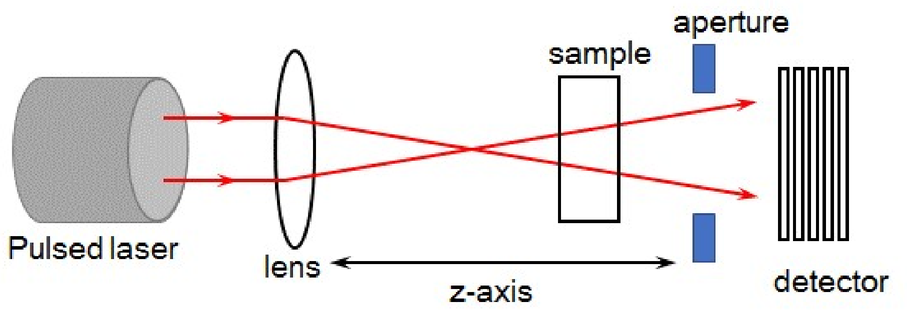

Fig. s2. Illustration of the experiment set for Z-scan.

Theoretically, the two-photon induced decrease of transmittance, T(z), a nonlinear medium under a single Gaussian pulse can be expressed as equations 1 and 2, and the two-photon absorption coefficient (β) can be obtained from the experimental data by fitting Z-scan curves to them.

$$T(\mathrm{z}) = \sum_{n=0}^{\infty} \frac{(-q)^n}{(n+1)^{3/2}} \qquad (1)$$

$$q = \frac{\beta I_0 L}{1 + z^2/z_0^2} \qquad (2)$$

Where n is an integer number from 0 to ∞, I_0 is the input intensity, L is the sample length, z is the sample position with respect to the focal plane, and z_0 is the diffraction length of the incident beam. According to the obtained β, the two-photon absorption cross-section (δ) can be calculated by the equation 3.

$$\delta = \frac{\beta \mathrm{h} \nu \times 10^3}{N_A C} \qquad (3)$$

Where $h\nu$ is photon energy, N_A is the Avogadro constant, and C represents the solute molar concentration. The unit (GM) of δ is normally shown as 1 GM = 10^{-50} cm^4 s $photon^{-1}$ $molecule^{-1}$.

The Z-scan technique is useful to probe nonlinear transmission and nonlinear refraction. However it should be caution that some effects caused by nonlinear scattering and excited-state population can tend to enhance the apparent TPA cross-sections during Z-scan.