Electronic Supplementary Information

Organic disulfide-modified folate carbon dots for tumor-targeted synergistic chemodynamic/photodynamic therapy

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Contents

1. PPa, DTPA drug loading capacity calculations for FCPPD

The absorbance of PPa was measured at different concentrations (0.05, 0.04, 0.03, 0.02, 0.01 μg/mL), and the first order linear equation of absorbance versus concentration was plotted (Figure S1a). The absorbance of DTPA was measured at different concentrations (0.5, 0.4, 0.3, 0.2, 0.1 μg/mL), and the first order linear equation of absorbance versus concentration was plotted (Figure S1b).

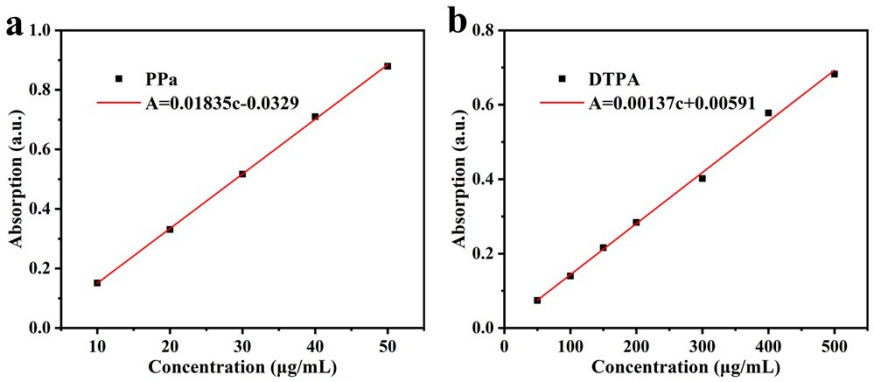

Figure S1. a) standard curves for PPa; b) standard curves for DTPA.

The experimental details are shown below:

10 mg of PPa, 3.2 mg of NHS, and 4.3 mg of EDC were dispersed into 3 mL of DMF and stirred at room temperature for 4 h. 30 mg of FCP-CDs were dispersed into 24 mL of deionized water. The above DMF solution was added slowly dropwise in FCP-CDs solution and stirred at room temperature for 48 h. The FCP-CDs@PPa were then purified by dialysis in ultrapure water using a dialysis bag (Mw = 1000) for 2 days.

10 mg of DTPA was dispersed into 3 mL of DMF, and 18.2 mg of NHS and 11 mg of EDC were added and activated by stirring at room temperature for 4 h. The above DMF solution was slowly added dropwise to FCP-CDs@PPa solution and stirred at room temperature for 48 h. The solid powder was obtained by freeze-drying and was

then purified by dialysis in ultrapure water using a dialysis bag (Mw = 1000) for 2 days to obtain FCP-CDs@PPa@DTPA (hereafter referred to as FCPPD).

During the preparation of FCP-CDs@PPa and FCP-CDs@PPa@DTPA, the solution outside the dialysis bag was collected at each dialysis step. The absorption values of PPa at 667 nm and DTPA at 243 nm were measured using UV-Vis absorption spectroscopy. After absorbance calculations were brought into the standard equation for conversion to drug mass, the drug loading rates of PPa and DTPA were calculated by the equation (experiments were repeated three times). The experimental data were recorded in Table S1 and Table S2. The following equations were used to calculate the drug loading rates:

$$Drug\ loading\ (\%) = \frac{m\ -\ m_1}{m_{total}} \times 100\ \% \quad (1)$$

where m is the mass of the drug initially put into the reaction, m_1 is the drug remaining outside the dialysis bag after loading, and m_{total} is the mass of FCP-CDs.

Table S1. Measured values of absorbance and load factor of PPa.

	A_{PPa}/OD	V_{PPa}/mL	m/mg	m_1/mg	m_{total}/mg	DLC/%
I	**0.121**	**242**	**10.02**	**0.202**	**30.04**	**32.7**
II	**0.132**	**224**	**10.01**	**0.201**	**30.03**	**32.7**
III	**0.125**	**235**	**10.03**	**0.202**	**30.04**	**32.7**
Average	**0.126**	**234**	**10.02**	**0.202**	**30.04**	**32.7**

Table S2. Measured values of absorbance and load factor of DTPA.

	A_{DTPA}/OD	V_{DTPA}/mL	m/mg	m_1/mg	m_{total}/mg	DLC/%
I	**0.011**	**237**	**10.01**	**0.881**	**30.04**	**30.4**
II	**0.011**	**245**	**10.02**	**0.910**	**30.03**	**30.3**
III	**0.011**	**239**	**10.02**	**0.888**	**30.04**	**30.3**
Average	**0.011**	**240.3**	**10.02**	**0.893**	**30.04**	**30.3**

2. Detections of singlet oxygen of FCPPD

1,3-Diphenylisobenzofuran (DPPF) has a characteristic absorption peak at 415 nm, which can react with $^1O_2$ and reduce the characteristic absorption peak so that DPPF can be used as a $^1O_2$ trapping agent. The $^1O_2$ quantum yields of PPa and FCPPD were evaluated using methylene blue (MB) as a standard control sample. The absorption peaks of the MB, PPa and FCPPD samples (670 nm, set to A_0) were adjusted by UV-Vis to 0.10 OD. 3 mL of the samples were placed in a quartz cuvette, 30 μL of DPBF (6×10^{-5} M in DMF) was added, and laser irradiation was performed at 10 s intervals from 0 to 100 s (λ = 655±20 nm, 40 mW/cm^2), and the absorbance (A_t) at 415 nm was measured. The standard curve was obtained with the DPBF degradation rate $ln_{At/A0}$ as the vertical coordinate and the time (t) as the horizontal coordinate. The quantum yield of $^1O_2$ was calculated using the following equation (2):

$$\Phi_s = \Phi_{MB}\frac{t_s}{t_{MB}} \quad (2)$$

where t_s and t_{MB} represent the time of DPBF degradation in the presence of sample and reference compound MB, respectively. Φ_{MB} is the $^1O_2$ quantum yield of MB (0.49).

Table S3. DPBF absorbance and degradation rate in different samples.

DPBF (containing MB）			DPBF (containing PPa)			DPBF (containing FCPPD)		
t_S	A	$ln(A_0/A_t)$	t_S	A	$ln(A_0/A_t)$	t_S	A	$ln(A_0/A_t)$
0	1.153	0	0	1.153	0	0	1.153	0
10	1.088	0.058	10	1.115	0.036	10	1.13	0.020
20	1.041	0.102	20	1.081	0.064	20	1.114	0.034
30	0.999	0.143	30	1.05	0.094	30	1.092	0.054
40	0.96	0.183	40	1.033	0.110	40	1.071	0.074
50	0.926	0.219	50	1.01	0.132	50	1.05	0.094
60	0.894	0.254	60	0.982	0.161	60	1.029	0.114
70	0.866	0.286	70	0.953	0.191	70	1.007	0.135
80	0.845	0.311	80	0.924	0.221	80	0.989	0.153
90	0.826	0.336	90	0.891	0.258	90	0.969	0.174
100	0.806	0.358	100	0.852	0.303	100	0.949	0.195

3. The MB contents at different mass concentrations and pH=5.5

Methylene blue (MB) is a characteristic ·OH indicator with a typical characteristic absorption peak at 665 nm. It can be selected as an indicator for monitoring hydroxyl radicals (·OH) as the absorption peak decreases gradually under the strong oxidizing effect of ·OH. 200 μL MB (10 μg/mL) was added to the PBS (pH=5.5) solution containing 200 μL H_2O_2 (100 mM) and 200 μL FCPPD (1 mg/mL) and incubated for 30 min at 37 °C. The changes in absorbance of MB at 665 nm were measured using a UV-Vis spectrophotometer. The experimental results were recorded in Table S4 and Table S5.

Table S4. The content of MB at pH=5.5.

MB (containing FCP-CDs)				MB (containing FCPPD)			
pH	**A**	**m_{MB} (μg)**	**W**	**pH**	**A**	**m_{MB} (μg)**	**W**
5.5	**0.873**	**17.86**	**19%**	**5.5**	**0.847**	**17.28**	**24%**
MB	**1.058**	**21.98**	**0%**	**MB**	**1.086**	**22.61**	**0%**

Table S5. The content of MB at different masses.

MB (containing FCPPD)				
pH	**A**	**m_{FCPPD} (μg)**	**m_{MB} (μg)**	**W**
5.5	**1.043**	**50**	**21.64**	**5%**
5.5	**0.95**	**100**	**19.52**	**14.6%**
5.5	**0.855**	**200**	**17.47**	**23.5%**
5.5	**0.713**	**300**	**14.05**	**38.6%**
MB	**1.0986**	**0**	**22.85**	**0%**

4. The Fenton-Like reaction mechanism of FCPPD

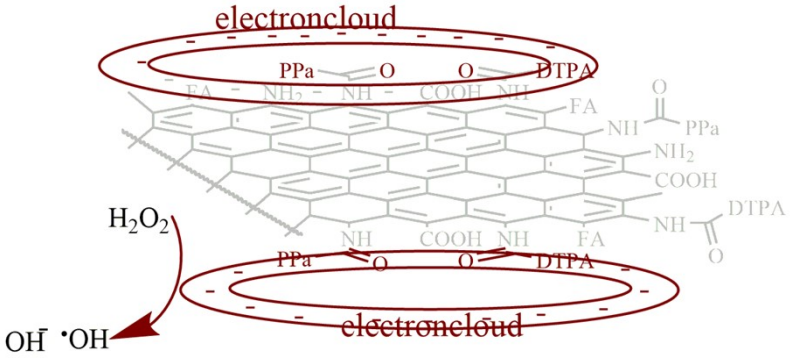

Figure S2. The Fenton-Like reaction mechanism of FCPPD.

5. Morphological changes of HeLa, 4T1 and L929 cells under FCPPD at different times

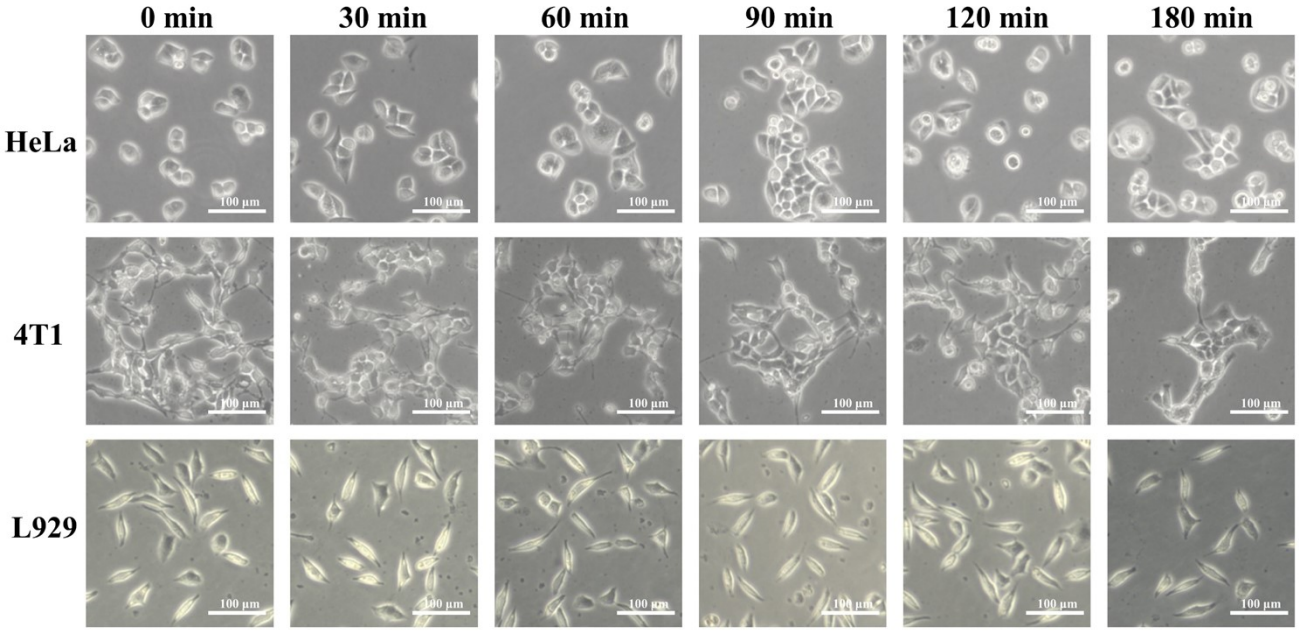

Figure S3. Morphological changes of HeLa, 4T1 and L929 cells under FCPPD at different times.

6. Fluorescence intensity of reactive oxygen species by PPa and FCPPD

Table S6. Fluorescence intensity of reactive oxygen species by PPa and FCPPD

HeLa (containing PPa）			HeLa (containing FCPPD)		
	Laser (min)	Intensity (a.u.)		Laser (min)	Intensity (a.u.)
	0	13915		0	21865
	5	25794		5	38765
	10	37261		10	48816
Control	0	11565	Control	0	12782

7. The synergy index of FCPPD chemodynamic/photodynamic therapy

The combined effect was assessed by calculating the synergy index of FCPPD chemodynamic/photodynamic therapy in different cells based on the response additivity approach [49]. The synergy index of FCPPD chemodynamic/photodynamic therapy was

calculated using the following equation:

$$Synergy\ index = \frac{E_A + E_B}{E_{AB}}$$

where E_A and E_B are the percentage of the apoptotic fraction induced by A (FCPPD) and B (PPa+Laser) alone, and E_{AB} (FCPPD+Laser) is the percentage of the apoptotic fraction induced by the combined treatment.

Synergy index values of less than 1 indicated a synergistic effect, synergy index values equal to 1 indicated an additive effect, and synergy index values of more than 1 indicated an antagonistic effect.

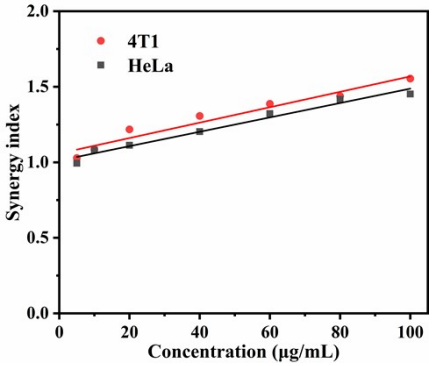

Figure S4. The synergy index of FCPPD chemodynamic/photodynamic therapy in different cells.