Supporting Information

pH Switchable Nano-Assembly for Imaging a Broad Range of Malignant Tumors

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Author Contributions

Y. L and Z. Q contributed equally to this work.

Experimental Section

The synthesis of compound DPP-thiophene-2

The mixture of DPP-thiophene-1 (3.00 g, 10.0 mmol), tert-butyl 6-bromohexylcarbamate (8.88 g, 30.0 mmol) and Cs_2CO_3 (16.2 g, 50.0 mmol) is dissolved into dimethylformamide (DMF, 60 mL) and stirred at 70 °C overnight. The mixture is concentrated in vacuum at 70 °C. The residue is washed with the mixed solvent of petrol ether and tetrahydrofuran (THF) (100 mL, petrol ether / THF = 1 / 1) to afford the crude product which is purified by silica gel column (the ratio between petrol ether / dichloromethane (DCM) is from 10 / 1 to 0 / 1), then DCM / methanol = 200 / 1 – 50 / 1) to afford DPP-thiophene-2 (4.00 g, yield 57%) as purple solid. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.83 (d, J = 3.6 Hz, 2H), 8.10 (d, J = 4.8 Hz, 2H), 7.41 (t, J = 4.8 Hz, 2H), 6.76 (t, J = 5.6 Hz, 2H), 3.99 (t, J = 7.2 Hz, 4H), 2.86-2.90 (m, 4H), 1.58-1.66 (m, 4H), 1.20-1.36 (m, 34H). MS (ESI) *m/z:* $[M - Boc + H]^+$ calcd for $C_{31}H_{43}N_4O_4S_2$ 599.27; found, 599.30.

The synthesis of compound DPP-thiophene-3

DPP-thiophene-2 (3.80 g, 5.44 mmol) dissolved in DCM (30 mL) is added into trifluoroacetic acid (TFA, 8 mL) dropwise at 0 °C. The reaction mixture is stirred at room temperature overnight, and is concentrated in vacuum at 40 °C. Methanol (50 mL) and K_2CO_3 (1.50 g, 10.9 mmol) is sequentially added into the concentrated product. The mixture is stirred at room temperature for 30 min, then concentrated in vacuum and purified by silica gel column (DCM / ammonia / methanol = 100 / 0.25 / 1 – 15 / 0.25 / 1) to afford DPP-thiophene-3 (2.50 g, yield 92%) as purple solid. ^{1}H NMR (400 MHz, MeOD-d_4, δ): 8.87 (dd, J1 = 4.0 Hz, J2 = 0.8 Hz, 2H), 7.94 (dd, J1 = 4.8 Hz, J2 = 0.8 Hz, 2H), 7.38 (t, J = 4.8 Hz, 2H), 4.15 (t, J = 7.2 Hz, 4H), 2.93 (t, J = 7.2 Hz, 4H), 1.75-1.84 (m, 4H), 1.64-1.72 (m, 4H), 1.44-1.53 (m, 8H). MS (ESI) *m/z:* $[M + H]^+$ calcd for $C_{26}H_{34}N_4O_2S_2$ 499.21; found, 499.20.

The synthesis of compound DPP-thiophene-4

The mixture of DPP-thiophene-3 (2.50 g, 5.02 mmol), K_2CO_3 (5.54 g, 40.2 mmol) and CH_3I (21.2 g, 151 mmol) is stirred at 60 °C overnight. The reaction mixture turns to red. Such mixture is cooled to room temperature and filtered to collect the precipitate. The cake is purified by C18 column* to afford DPP-thiophene-4 (1.80 g, yield 48%) as purple solid. ^{1}H NMR (400 MHz, MeOD-d_4, δ): 8.75 (dd, J1 = 4.0 Hz, J2 = 0.8 Hz, 2H), 7.84 (dd, J1 = 5.2 Hz, J2 = 1.2 Hz, 2H), 7.26 (dd, J1 = 4.8 Hz, J2 = 4.0 Hz, 2H), 4.00 (t, J = 7.6 Hz, 4H), 3.21-3.29 (m, 4H), 3.20 (s, 18H), 1.65-1.74 (m, 8H), 1.35-1.42 (m, 8H). MS (ESI) *m/z:* 1/2[M - 2Br]$^+$ calcd for $C_{16}H_{24}N_2O_1S_1$ 292.16; found, 292.30.

*Prep-HPLC Method Information:

Column: C18, 120 g, 20-35 μm; Flow Rate (mL min^{-1}): 40; Phase A: H_2O (aq. HBr, 0.2%), Phase B: methanol.

Gradient Program:

Time (min)	From (phase B) %	To (phase B) %
10	0	0
20	0	10
30	10	30
50	30	70
60	70	100
70	100	0

The detection of physiological indicators

DPP-thiophene-4 (2.5 μg) is intravenously injected per mice (n=6). After 24 hours, Mice blood samples are collected. Normal mice is used as control. Eleven physiological indicators in mouse blood, including white cells, red cells, platelet, hemoglobin, albumin, protein total, urea, uric acid, alanine aminotransferase, aspartate transaminase and alkaline phosphatase, are detected by routine blood examination instrument (Coulter-JT) and biochemical detector (Roche cobas 6000).

Supporting Figures

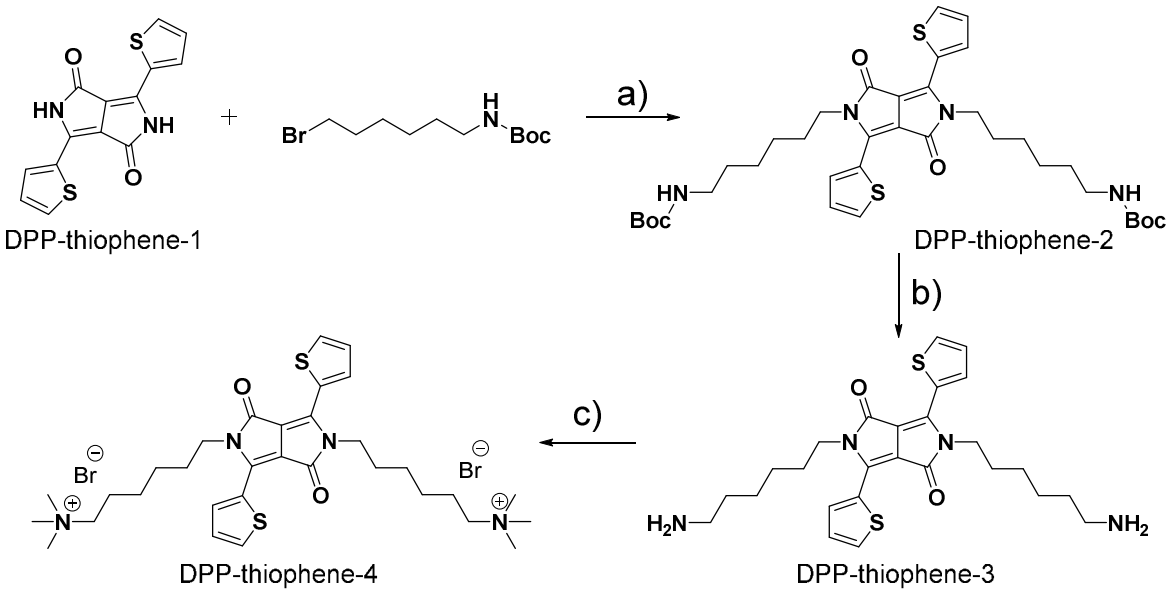

Figure S1. The synthesis route of DPP-thiophene-4. Reagents and conditions: a) Cs_2CO_3, DMF, 70 ^oC, overnight, 57%; b) TFA, 0 ^oC-RT, overnight, 92%; c) K_2CO_3, CH_3I, 60 ^oC, overnight, followed by RP-HPLC with 0.1% HBr, 48%. DMF = dimethylformamide, TFA = trifluoroacetic acid.

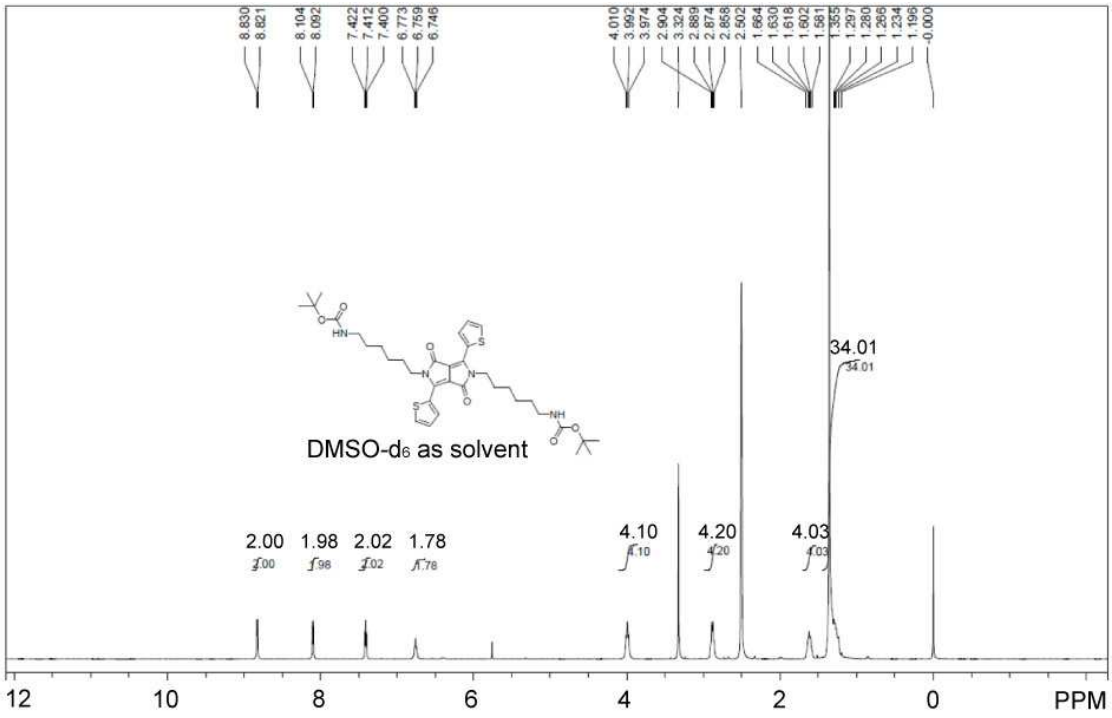

Figure S2. ^{1}H NMR of compound DPP-thiophene-2.

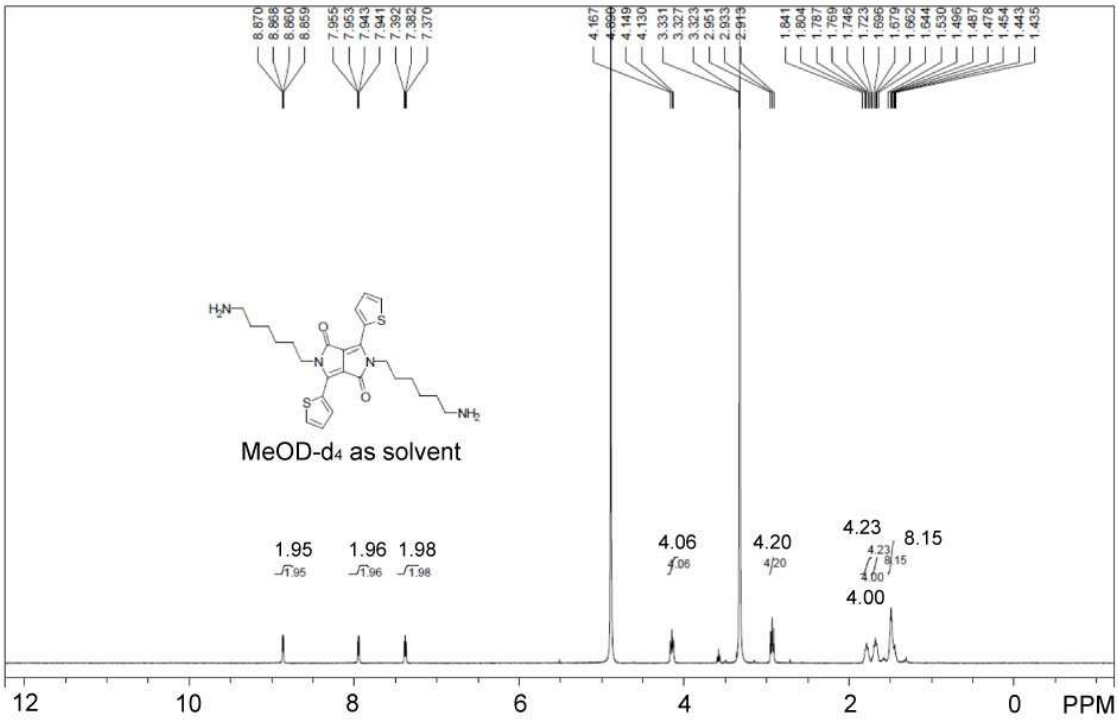

Figure S3. ^{1}H NMR of compound DPP-thiophene-3.

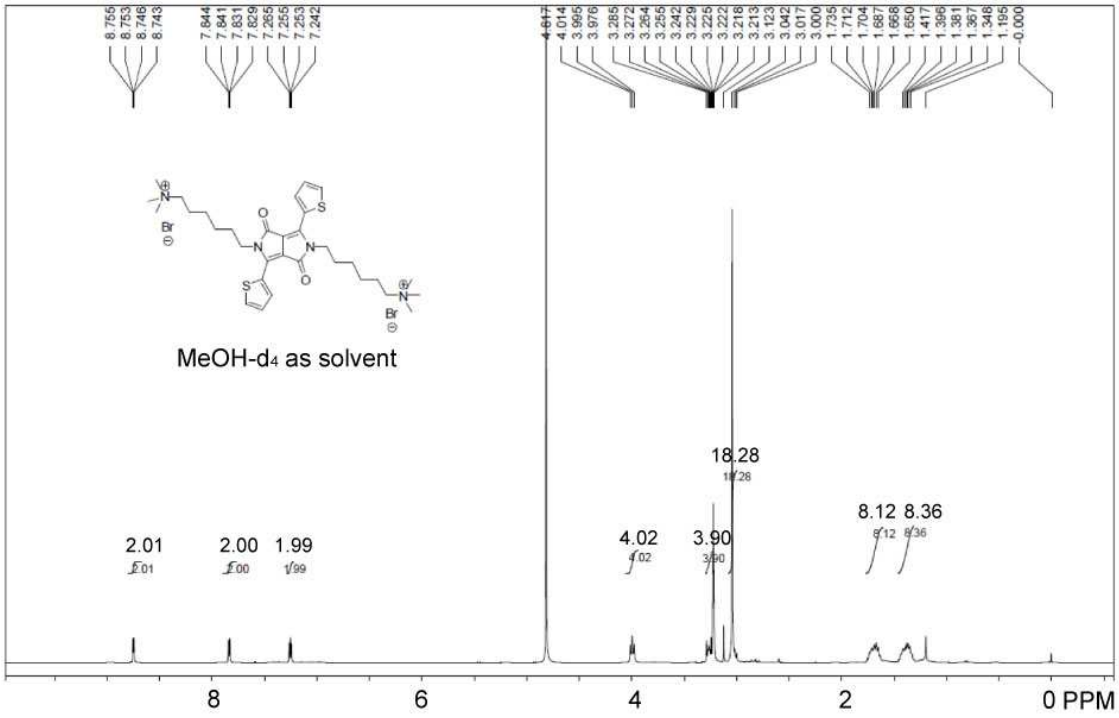

Figure S4. ^{1}H NMR of compound DPP-thiophene-4.

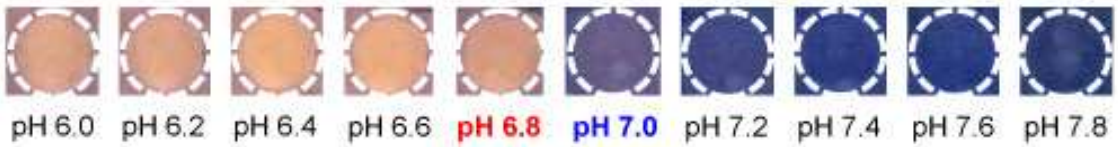

Figure S5. The fluorescent images of DPP-thiophene-4 (10 μg/ml) from pH 6.0 to pH 8.0. White dotted circles show fluorescent areas.

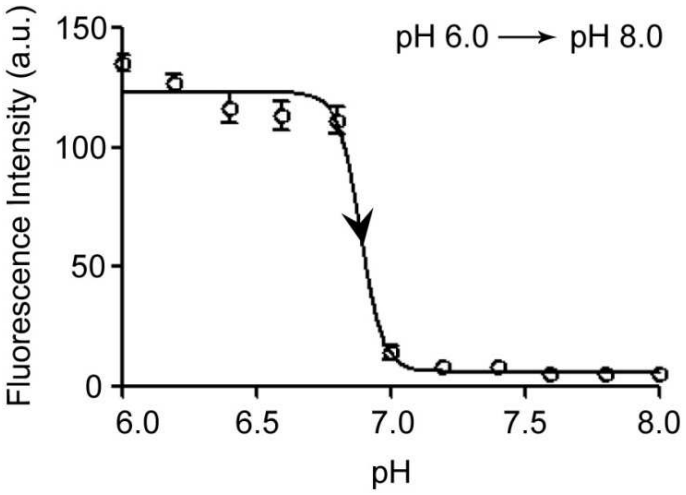

Figure S6. The fluorescence of DPP-thiophene-4 sharply decreases when the pH value of solution is reversely increased above 7.0.

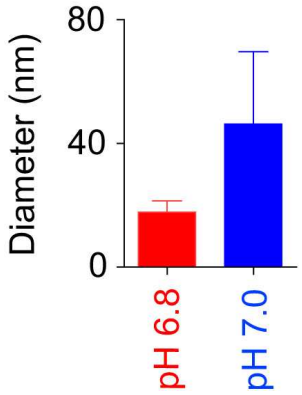

Figure S7. Based on TEM images of DPP-thiophene-4 at either pH 6.8 or pH 7.0，we calculate the diameter of nano-assembly at either pH 6.8 or pH 7.0 *via* randomly measuring 100 nano-assemblies.

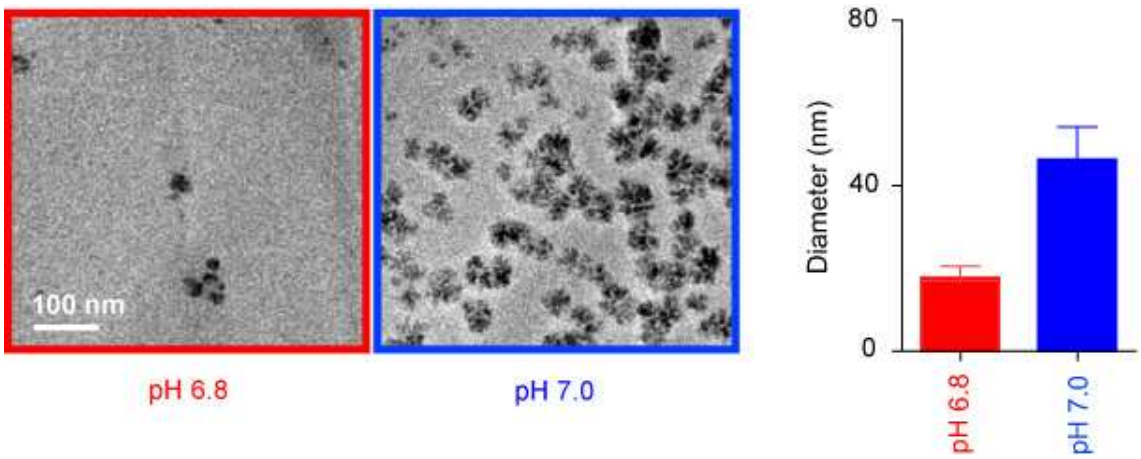

Figure S8., TEM images are obtained by the vortex of DPP-thiophene-4 solutions (pH 6.8 and 7.0) for 10 minutes. Nano-assemblies appear at pH 7.0 whereas nano-assemblies dis-assemble once the pH slightly decreases to 6.8. We calculate the diameter of nano-assembly at either pH 6.8 or pH 7.0 *via* randomly measuring 100 nano-assemblies in TEM images.

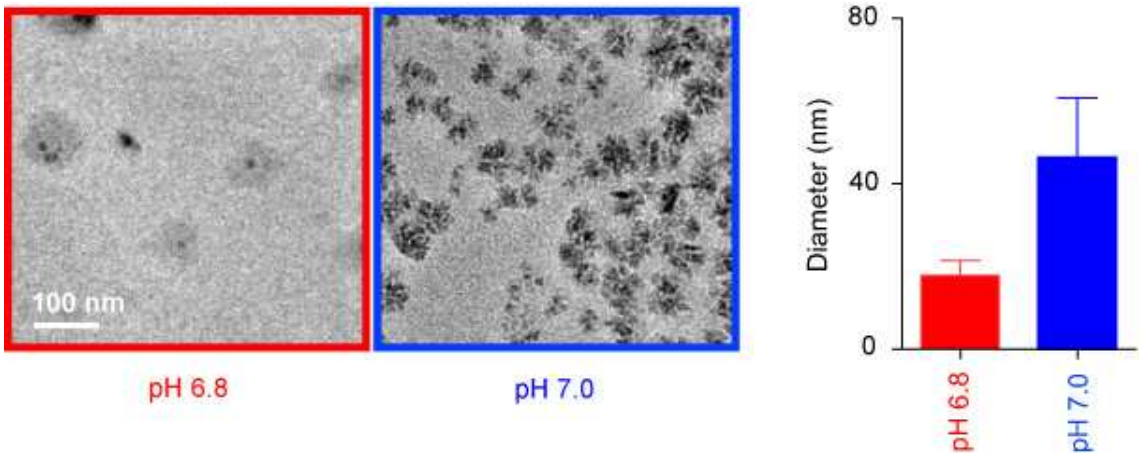

Figure S9. Staying DPP-thiophene-4 solutions (pH 6.8 and 7.0) in 50 ^{o}C for 30 minutes, nano-assemblies appear at pH 7.0 whereas nano-assemblies dis-assemble once the pH slightly decreases to 6.8. We calculate the diameter of nano-assembly at either pH 6.8 or pH 7.0 *via* randomly measuring 100 nano-assemblies in TEM images.

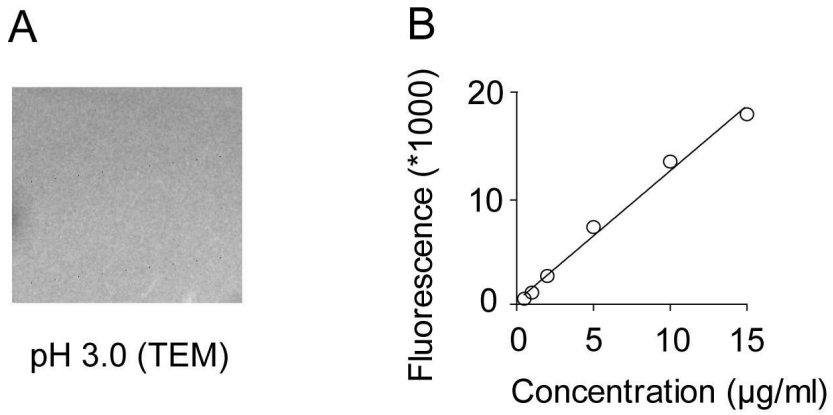

Figure S10. (A), (B) DPP-thiophene-4 molecules are fully dissolved at pH 3.0 (TEM). It shows a good linear relationship between the concentration (0-15 μg/ml) and fluorescence intensity of DPP-thiophene-4 (right).

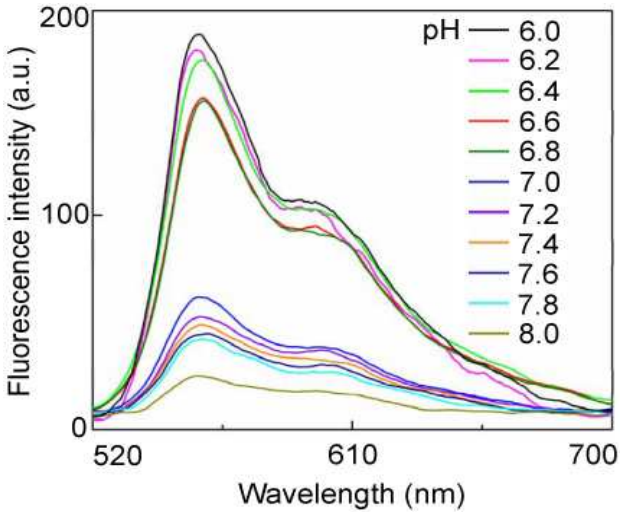

Figure S11. The fluorescence intensity of DPP-thiophene-4 in different pH solutions (pH 6.0-8.0).

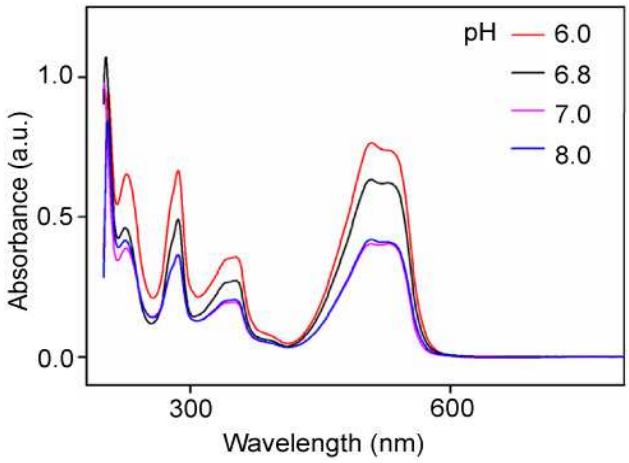

Figure S12. The ultraviolet absorption of DPP-thiophene-4 in different pH solutions (pH 6.0-8.0).

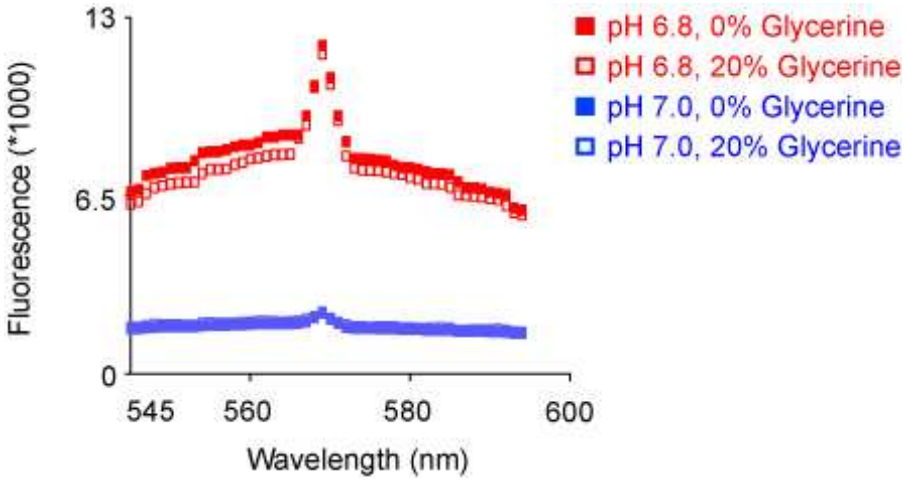

Figure S13. Under either pH 6.8 or 7.0, the fluorescence intensity of DPP-thiophene-4 solution with 20% glycerine (volume ratio) does not significantly change, compared with that of glycerine-free DPP-thiophene-4 solution.

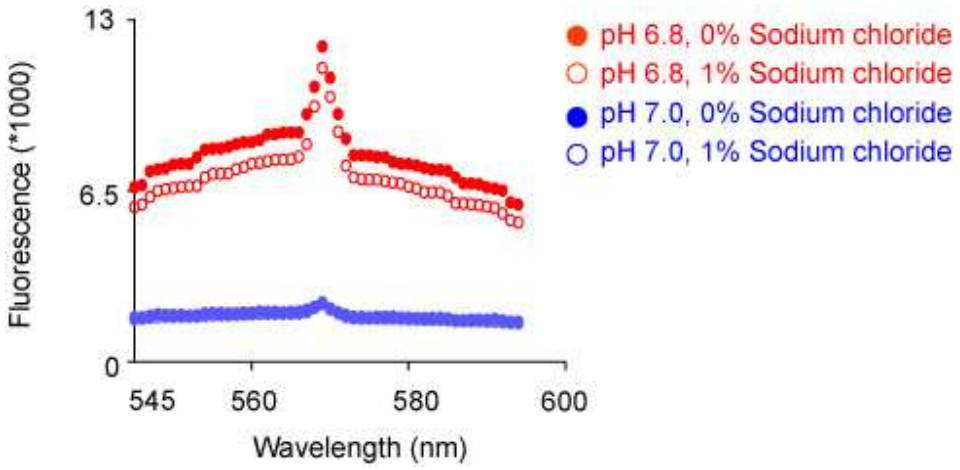

Figure S14. The fluorescence intensity of DPP-thiophene-4 solution with 1% sodium chloride (weight ratio) does not significantly change under either pH 6.8 or 7.0, compared with that of naked DPP-thiophene-4 solution.

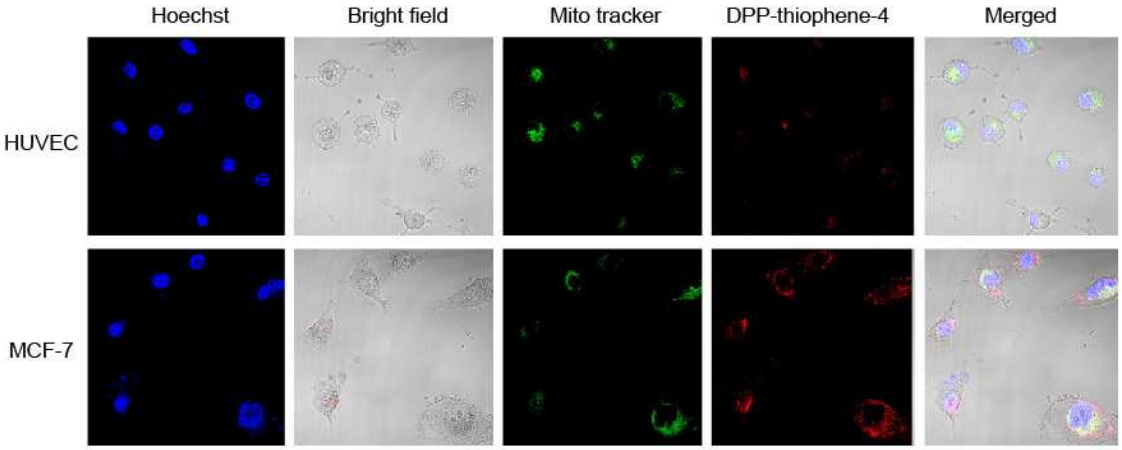

Figure S15. The overlap of DPP-thiophene-4 stained organelle and MitoTracker Green FM dye stained mitochondria in tumor cells (MCF-7) and normal cells (HUVEC). For each cell line, 1×10^6 above 90% vitality cells are cultured with 10 μg/ml DPP-thiophene-4 (red), 50 nM MitoTracker Green FM dye (green) or 10 μg/ml Hoechst 33342 (blue) for 20 minutes. The experiment is detected by confocal microscopy (Zeiss) at 543 nm excitation for DPP-thiophene-4, 490 nm excitation for MitoTracker Green FM dye, 352 nm excitation for Hoechst 33342.

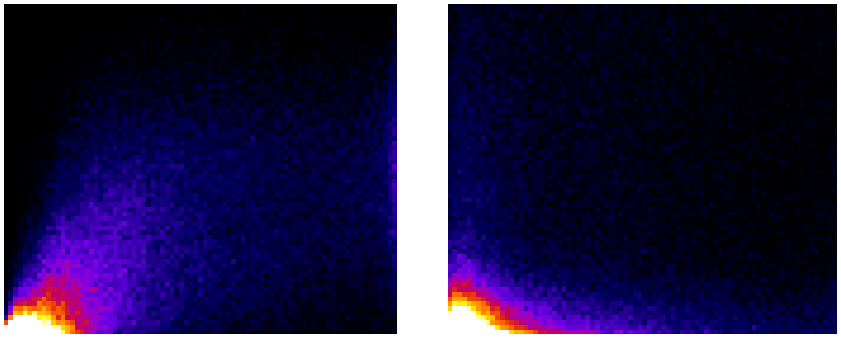

Figure S16. The Pearson's Correlation plots of DPP-thiophene-4 with Lyso Tracker Green DND-26 (left) or MitoTracker Green FM dye (right) in MCF-7 cells.

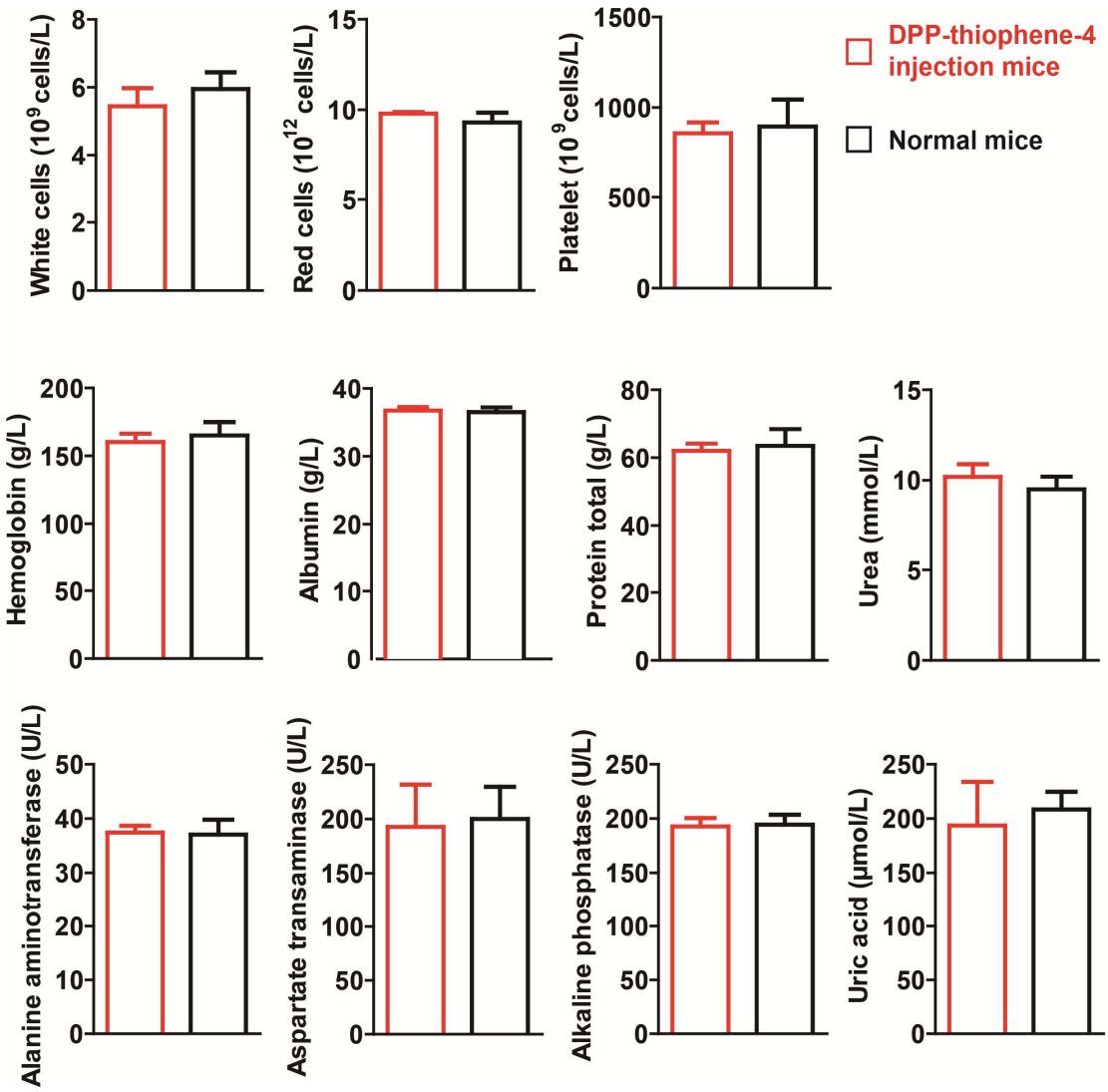

Figure S17. The effects of DPP-thiophene-4 on blood cells and physiological index in mice. DPP-thiophene-4 (2.5 μg) is intravenously injected per mice (n=6). After 24 hours, the blood samples are collected. Normal mice is used as control. The experiments are repeated twice.

Table S1. Comparison of pH responsiveness and the ease of preparation of various typical pH-sensitive probes.

Probes	**Δ F. I.** [a]	**Ratio of Δ F. I./Δ pH** [b]	**Component**	**Ease of Preparation**	**Ref**
DPP-thiophene -4	> 10 folds (pH 6.8-7.0)	> 50	Small molecule	Easy, controllable	
16 types of peptidic probes	~ 5 folds (pH 2-8)	< 1	Peptide	Easy, controllable	1
Micellar probe	> 50 folds (pH 6.8-7.1)	> 160	Organic polymer	Relatively difficult	2
Boron-dipyrro methene fluorophore	> 50 folds (pH 4.0-8.0)	> 12	Small molecule	Easy, controllable	3
Nanoparticle	~ 100 folds (pH 6.7-7.4)	~ 140	Organic polymer	Relatively difficult	4, 5
LysoTracke Green DND-26 (commercial)	> 10 folds (pH 4.0-6.0)	> 5	Small molecule	Easy, controllable	6
LysoSensor Yellow/Blue DND-160 (commercial)	> 10 folds (pH 3.0-8.0)	> 2	Small molecule	Easy, controllable	7, 8

Note: [a] Δ F. I.: the changed fluorescence intensity of probe in the pH interval; [b] Ratio of Δ F. I./Δ pH is the value of Δ Fluorescence intensity /Δ pH.

References

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