Electronic Supplementary Information for

Engineering dual-responsive, exosome-surface anchored DNA nanosensor for monitoring of microenvironment in vivo

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1. Experimental Section:

1.1 Materials and instruments.

Ultrapure water obtained from Millipore water purification system (18 MΩ) was used in all assays. Azoreductase was purchased from CHI Scientific (Jiangsu China). Nicotinamide adenine dinucleotide phosphate (NADPH) was purchased from Beyotime (Shanghai, China). All DNA oligonucleotides were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). The sequences of the oligonucleotides used in this experiment are described in Table S1. Phosphate-buffered saline (PBS, 0.01 M) solutions with various pH values were obtained by mixing 0.1 M $Na_2HPO_4 \cdot 12H_2O$ and 0.1 M $NaH_2PO_4 \cdot 2H_2O$ in different ratios and following by adding 4.6 mM KCl, 5 mM $MgCl_2$, and 130 mM NaCl. The small pH perturbation was adjusted by 0.1M NaOH or 0.1M HCl. The Tris-borate-EDTA (TBE) buffer was prepared by adding 89 mM Tris, 89 mM boric acid and 2 mM EDTA. Fetal bovine serum (FBS), 1640, penicillin-streptomycin and PBS were purchased from Beijing Dingguo Changsheng Biotechnology Co., Ltd.

Fluorescence emission spectra were measured on a PTI QM4 fluorescence system (Photo Technology International, Birmingham, NJ) with accessories of a temperature controller. Circular dichroism (CD) spectra in the experimental data were obtained on a MOS-500 spectrophotometer (Claix, France). Dynamic light scattering (DLS, ZEN3690, Malvern Corp, U.K.) was carried out to analyze the size and zeta potential of exosomes and TDPE. All fluorescence images of cells were obtained using an Olympus FV1000-MPE multiphoton laser scanning confocal microscope (Japan). All the chemicals were of analytical grade and used without further purification. Sartorius ultrapure water was used throughout the experiments.

1.2 Fabrication of nanospring and TDP

Four customized single-strand oligonucleotides (L1-4) were first mixed in a PBS buffer (pH 7.4) at a molar ratio of 1:1:1:1 to achieve a concentration of 2 μM. The mixture was heated at 95 °C for 5 min in a water bath and then naturally cooled to room temperature to obtain DNA nanospring. L1 was replaced with an I-strand

(without BHQ2) and then obtained UB-nanospring through the same preparation process.

Four DNA oligonucleotides (S1-3-cho and S4) and nanospring strand were mixed together in a PBS buffer (pH 7.4) at a molar ratio of 1:1:1:1 and the final concentration of each oligonucleotide was 2 μM. The mixture was slowly annealed from 95 ℃ to room temperature (RT). The resultant DNA products were stored at 4 ℃ for further use.

1.3 Exosome extraction

Exosomes were collected using conventional centrifugation from supernatant media of ScaBER cells. Cells were cultured in T182 cm^2 flasks in exosome-depleted FBS supplemented DMEM until they reached a confluency of 80~ 90%. Cell culture media were collected and centrifuged at 300 g for 10 min at 4 °C. Then, the supernatant was centrifuged at 2,000 g for 10 min at 4 °C to discard cellular debris, followed by filtration using a 0.22 μm filter (vacuum-driven filter, 25-229, Genesee Scientific). The filtered media were then ultracentrifuged at 27,000 rpm for 2 h at 4 °C. Pellet was pipetted and washed with 36 mL PBS, followed by another centrifugation at 27,000 rpm for 2 h at 4 °C. Finally, the supernatant was discarded, and exosomes were resuspended in 100 μL PBS. The collected exosomes were stored at -80 °C.

1.4 Synthesis of TDPE

1 μM TDP was incubated with exosomes at 4 ℃ for 45 minutes for cholesterol anchoring. The TDPE formed was then collected by centrifugation at 100000 g for 70 minutes at 4°C.

1.5 Gel electrophoresis

To characterize the construction of DNA tetrahedron structures, each DNA sample (10 μL, 1 μM) was mixed with 6× loading buffer (2 μL). Electrophoresis was performed in 1×TBE buffer at 100 V for 60 min. After that, the polyacrylamide gel was imaged on a Bio-Rad molecular imager under UV light. To assess the structural stability, ROX-labeled DNA tetrahedrons (1 μM) and nanosprings were incubated in

fresh 1640 medium plus 10% FBS at 37 ℃ for different time spans. Then, 10 μL of the sample was mixed with glycerin (2 μL). Electrophoresis was performed in 1×TBE buffer at 110 V for 30 min. Finally, gels were imaged on a Bio-Rad molecular imager under UV light.

1.6 Fluorescence spectroscopy assay

For fluorescence detection of pH, the constructed nanospring (2 μM) , TDP (2 μM) or TDPE was dispersed in different pH PBS buffer. For fluorescence detection of oxygen, the nanospring was dispersed in PBS buffer, followed by addition of AzoR and NADPH (50μM) under hypoxic conditions (1% O_2). Fluorescence emission intensity was recorded from 590 to 650 nm with excitation set at 570 nm for pH assay, and 650 to 750 nm with excitation set at 635 nm for oxygen assay.

The limit of detection was calculated using the following formula: LOD = 3.3 σ/S, where σ is the standard deviation of y-intercept of the regression line and S is the slope of the calibration curve.

1.7 CD measurements

DNA probes (75 μL, nanospring) were added to 225 μL of PBS with different pH values, followed by incubation for 30 min at 37 °C. Then, we recorded the CD spectra of each DNA probe at 220–340 nm. Three scans were performed and averaged after subtracting the background of the corresponding PBS solution. The final concentration of nanospring was 0.5 μM.

1.8 Cell culture.

ScaBER cells were grown in fresh 1640 supplemented with 10% inactivated fetal bovine serum, 100 U/mL, 1% penicillin and streptomycin solution maintained in a humidified CO_2 incubator with 5% CO_2 at 37 °C.

1.9 Confocal fluorescence imaging

ScaBER cells were cultured in plastic culture dishes for 24 h and then washed with D-PBS for three times. Then, ScaBER cells were trypsinized for one minute firet and then incubated with TDP treated with or without rat liver microsomes in D-PBS (100 nM, 200 μL) at RT for 10 min. After three times washes with PBS, the cells

were placed in PBS buffer with various pH values (pH 5.5 - 7.4) for imaging. Imaging was performed with a confocal laser-scanning microscope (Nikon, Japan) with a 20× objective lens.

The co-localization of DiO and TDP was to add DiO and incubate for 20 minutes after the cells were incubated with TDP. After three times washes with PBS, the cells were placed in PBS buffer (pH 7.4) for imaging.

For pH responsive imaging in living cells, ScaBER cells were seeded in confocal dishes and cultured in CO_2 incubator for 24 h, then incubated with Rho-TP for 2h.[1] Before imaging, cells were washed with PBS three times and treated with high K^+ buffer solution (20 mm NaCl, 125 mm KCl, 0.5 mm $CaCl_2$, 0.5 mm $MgCl_2$, 5.0 mm glucose, and 20 mm HEPES, containing 10 μM nigericin) with various pHs (5.5-8,5), which is a standard approach for homogenizing the pH of cells and culture medium.[2] The fluorescence signal was recorded at 570-650 nm with excitation at 559 nm.

The fluorescence imaging was performed on an Olympus FV1000-MPE multiphoton laser scanning confocal microscope equipped with 20X objective and 60X objective. ROX was excited with the 559 nm laser, and its fluorescence signal was collected through emission channel of 570 - 625 nm. Cy5 was excited with the 640 nm laser, and its fluorescence signal was collected through emission channel of 655 - 755 nm. The emission wavelength of DiO was set as 490-520 nm, and its excitation wavelengths were set as 488 nm. The data were analyzed with image J.

1.10 Transmission electron microscopy

The size of exosome was characterized by transmission electron microscopy (TEM). Sample was loaded on TEM copper grits and dried for 2 hours. After washing with water twice, sample was negatively stained by 2% phosphotungstic acid. The dried sample was observed on a Hitachi H-7000 NAR transmission electron microscope using a working voltage of 100 kV.

1.11 Fluorescence imaging study in vivo

For in vivo imaging, the tumor xenograft model was built by injecting 4T1 cells (1×10^6 cells/100 μL in 1:1 (v/v) PBS and Matrigel) subcutaneously into the right flank of nude mice. The tumor volume (V) was calculated using the following

equation: V = length × width^2 /2. When the tumors grew to 100-400 mm^3, TDPE (DNA probe dose of 1 μmol/kg) was injected into the tumor and non-neoplastic regions of 4T1 tumor-bearing mice. In order to simulate the areas of inflammation and hypoxia after exercise, the mice were pretreated with LPS (2 mg/mL) and $CoCl_2$ (2 mg/mL). Mice were anesthetized with isoflurane and imaged upon 1, 30, 60, and 120 min postinjection. The fluorescence images of live mice were collected with an IVIS Lumina XRMSIII in vivo imaging system (Caliper Life Science, USA).

2. Experimental data

Table 1 abbreviations

abbreviations	full name
AGE	Agarose gel electrophoresis
AzoR	Azoreductase
BHQ2	the black hole quencher 2
CLSM	Confocal fluorescence images
Cy5	Cyanine dyes 5
ROX	Carboxy-x-rhodamine
DLS	Dynamic Light Scattering
DNA	Deoxyribonucleic acid
GSH	Glutathione
H_2AzDc	azobenzene-4,4'-dicarboxylic acid
MTT	3-(4,5)-dimethylthiahiazo (-z-y1)-3,5-di- phenytetrazoliumromide
NADPH	beta-nicotinamide adenine dinucleotide phosphate tetrasodium salt (reduced form)
O_2	Oxygen
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffer saline
PsC	Pearson coefficient
ScaBER	Human bladder cancer cells
TDP	A pendant-shaped tetrahedral structure with nanosprings connected to one vertex
TDPE	exosome-surface anchored-DNA nanospring
TEM	transmission electron microscope

Table 2 Oligonucleotides Sequences

Name	Sequence (5 '--3 ')
L1:	AAC GGT CGC TAC AGA TTT(BHQ2) CCCCCCT CCCCCC TTTA CCCCCCT CCCCCC TTT CGC AGT CCA GAG AGG
L2:	AGA GAA CCT GGG CTC CCT CTC TGG ACT GCG (ROX)
L3:	GGTAAAGCGGGCAGGTGGG
L3-1	AGGGGGG TAAA GGGGGG A
L3-2	ACCCCGG TAAA CCGGCC A
L3-3	GGTAAATGGGTGAGTGGGG-
L4:	(Cy5)TCT GTA GCG ACC GTT
L1(without BHQ2):	AAC GGT CGC TAC AGA TTT CCCCCCT CCCCCC TTTA CCCCCCT CCCCCC TTT CGC AGT CCA GAG AGG
S1:	TAT CAC CAG GCA GTT GAC AGT GTA GCA AGC TGT AAT AGA TGC GAG GGT CCA ATA CTT
S2:	TCA ACT GCC TGG TGA TAA AAC GAC ACT ACG TGG GAA TCT ACT ATG GCG GCT CTT CTT
S3:	TTC AGA CTT AGG AAT GTG CTT CCC ACG TAG TGT CGT TTG TAT TGG ACC CTC GCA TTT
S4:	GAG CCC AGG TTC TCT TTTTTT ACA TTC CTA AGT CTG AAA CAT TAC AGC TTG CTA CAC GAG AAG AGC CGC CAT AGT A -3'
S1-cho:	TAT CAC CAG GCA GTT GAC AGT GTA GCA AGC TGT AAT AGA TGC GAG GGT CCA ATA CTT-cholesterol
S2-cho:	TCA ACT GCC TGG TGA TAA AAC GAC ACT ACG TGG GAA TCT ACT ATG GCG GCT CTT CTT-cholesterol
S3-cho:	TTC AGA CTT AGG AAT GTG CTT CCC ACG TAG TGT CGT TTG TAT TGG ACC CTC GCA TTT-cholesterol

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Rat Liver Microsomes

Cat. No: 6-1002

Batch Number 21FS012L

Description of Production

Species: Rat	Volume: 0,5mL
Strain: Sprague Dawley	Protein Concentrations 20 mg/ml.
Gender: Male	Storage Bufier:0.25M Sucrose
Age: 6-8 weeks	Storage Conditions: -80°C
weight 200-220 g	Deliver Conditions: Dry lee
Tissue: Liver	Control Date Dec,5.2021
Pool 50 Untreated	Expiry Date: Dec,4, 2022

Specific Content and Enzyme Activities	**Content /Rate**
Cytochrome P450 content (nmol/mg protein)	0.806
Cytochrome b5 content (nmol/mg protein)	0.402

Enzyme	Reaction	[s](μM)	Rate (pmol/mg/min)
CYP2E1	Rate of formation of Chlorzoxazone 6-hydroxylation	300	2214
CYP3A4/5	Rate of formation of 6β-hydroxy Testorsterone	200	4041

To measure Cytochrome P450 (CYP) activity, liver microsomes (0.1 mg/ml) were incubated in triplicate at 37°C phosphate buffer (50 mM, pH 7.4.containling $MgCl_2$ (4.0 mM), NADP (1.0 mM), glucose-6-phosphate (10.0mM), glucose-6- phosphate dehrydrogenase (1 Unit/mL). EDTA (1.0 mM) and marker substrate ,at the final concentration indicated. Metabolite formation was determined by validated HPLC methods win deuterated metabolites as internal standards.

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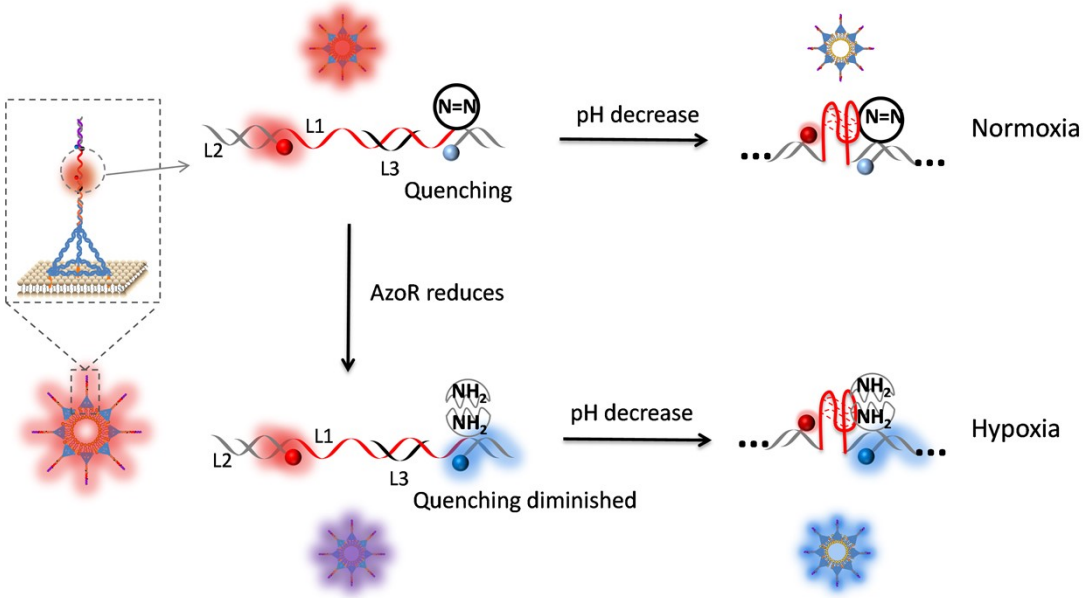

Scheme S1: Schematic illustration of TDP conformational changes in response to O_2 reduces and pH decrease.

Scheme S2: The formation process of TDPE

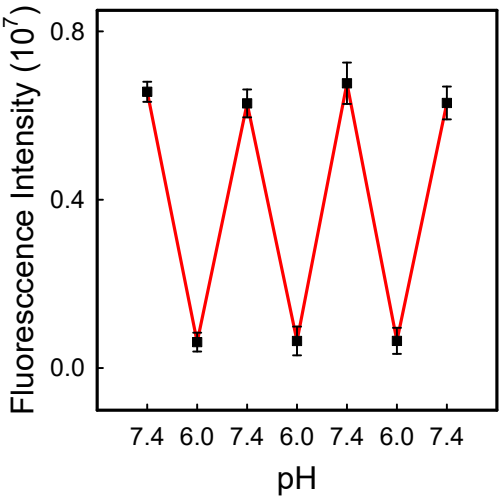

Fig. S1 (A) The background of CD spectroscopy. (B) CD spectra of TDP in 10 mM PBS buffer at pH 5.5 and pH 7.4. The concentrations of TDP was 0.5 μM

Fig. S2 Optimization of L3-strand sequences: A, C and E represent the representative fluorescence spectra of 100 nm nanaospring in various pH solutions using sequences L3-1, L3-2 and L3-3, respectively. The B, D and F represent normalized ROX fluorescence intensity of 100 nM nanospring as a function of pH in the buffer (the fluorescence intensity of ROX obtained at pH 7.4 was normalized to 1.0)of L3-1, L3-2 and L3-3, respectively. Error bars represent variations between three measurements.

Fig. S3 The reversibility of 100 nM nanospring demonstrated by the ROX fluorescence intensity to pH changes between 7.4 and 6.0, repeatedly. λex = 570 nm. λem = 607 nm. Error bars represent variations between three measurements.

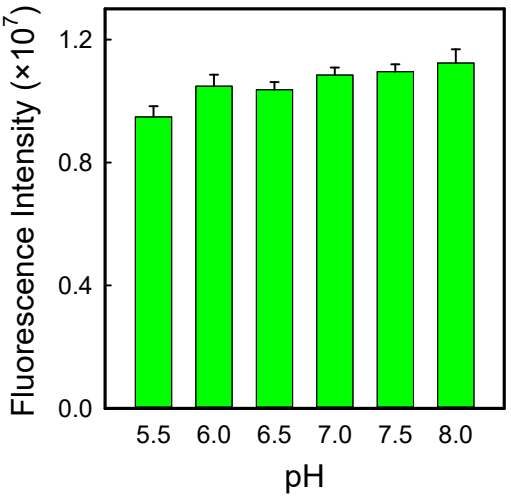

Fig. S4 Fluorescence intensity stability of ROX collected from 100 nM nanospring at different pH values (pH 5.5-8.0). λex = 570 nm. λem = 607 nm. Error bars represent variations between three measurements.

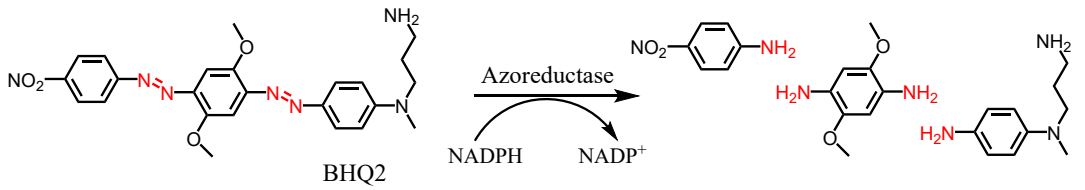

Fig. S5 (A) Schematic diagram of the BHQ2 responses to azoreductase. (B) FTIR spectra of (a) H_2AzDc (4,4'-azobenzene dicarboxylic acid, as a model to investigate the hypoxia-sensitive structural transformation of the azo group in BHQ2), H_2AzDc upon incubated with 100 μg·mL^{-1} rat liver microsomes, with the aid of NADPH (50 μM) at 37°C for 4 h under 1% O_2 (except for the different concentrations of rat liver microsomes, other conditions are the same in the following text), (b) the wavenumber located at 1440-1410 cm^{-1} was assigned to the N=N stretching in the compounds of trans aromatic azo. All 1% O_2 conditions were mimicked by bubbling nitrogen gas through the reaction mixture.

Fig. S6. (A) Representative fluorescence emission spectra of 100 nM nanospring under different conditions. Black curve: the nanospring in PBS buffer (pH 7.5; black curve); Color curve: the nanospring in the presence of rat liver microsomes (100 μg·mL^{-1}) and NADPH (50 μM) at 37°C for 4 h under 1% O_2 (except for the different concentrations of rat liver microsomes, other conditions are the same in the following text) in buffer at pH 5.5-8.0. λex = 635nm. (B) Fluorescence enhancement (F/F_0, where F represent the fluorescence intensity of nanospring upon incubated with rat liver microsomes (100 μg·mL^{-1}) and 50μM NADPH addition under 1% O_2, and F_0 represent the fluorescence intensity of nanospring without adding rat liver microsomes and 50 μM NADPH) as functions of pH. λex = 635nm. λem = 667 nm. Error bars represent variations between three measurements. All 1% O_2 conditions were mimicked by bubbling nitrogen gas through the reaction mixture.

Fig. S7 (A) Representative fluorescence emission spectra and (B) fluorescence emission intensity of Cy5 collected from 100 nM nanospring upon incubated with different concentrations of rat liver microsomes (0, 20, 40, 60, 80 and 100 μg·mL^{-1}) and NADPH (50 μM) at 37 °C for 4 h under 1% O_2. λex = 635 nm. (C) Representative fluorescence emission spectra of Rho-TP (10μM) with different concentrations of rat liver microsomes (0, 20, 40, 60, 80 and 100 μg·mL^{-1}) and NADPH (50 μM) at 37 °C for 4 h under 1% O_2. λex = 560 nm. (D) Representative fluorescence intensity stability of Cy5 collected from 100 nM UB-nanospring at different pH values (pH 5.5-8.0). λex = 635 nm. Error bars represent variations between three measurements.

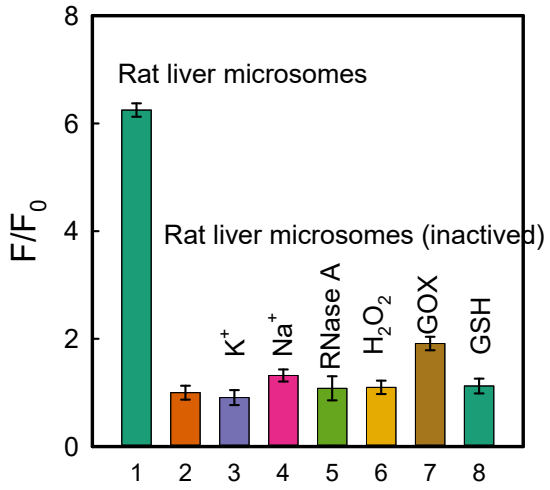

Fig. S8 Fluorescence emission intensity changes of 100 nM nanospring at pH 7.4 in the presence of diverse metal ions and bioactive small molecules: rat liver microsomes (active or inactived) (100 μg·mL^{-1}); K^+ (10 mM), Na^+ (10 mM), RNase A (10 ng/μL), H_2O_2 (10 mM), GOX (10 μM), GSH (10 mM). F and F_0 represent the fluorescence emission intensity of nanospring after and before the addition of azoreductase or other interferential species. λex = 635 nm. λem = 667 nm. Error bars represent variations between three measurements.

Fig. S9. (A) Representative fluorescence emission spectra of 100 nM nanospring after incubated with 50 μg·mL^{-1} rat liver microsomes and NADPH (50 μM) at 37 ℃ for 4h

C)

under 1% O_2 in buffer solution at pH 5.5-7.4. (B) Normalized ROX fluorescence intensity of 100 nM nanospring as a function of pH after incubated with 50 μg·mL^{-1} rat liver microsomes and NADPH (50 μM) at 37 ℃ for 4h under 1% O_2 (the fluorescence intensity of ROX obtained at pH 7.4 was normalized to 1.0). λex = 570 nm. (C) Representative fluorescence emission spectra of 100 nM nanospring after incubated with 100 μg·mL^{-1} rat liver microsomes and NADPH (50 μM) at 37 ℃ for 4h under 1% O_2 in buffer solution at pH 5.5-7.4. (D) Normalized ROX fluorescence intensity of 100 nM nanospring as a function of pH after incubated with 100 μg·mL^{-1} liver microsomes and NADPH (50 μM) at 37 ℃ for 4h under 1% O_2 (the fluorescence intensity of ROX obtained at pH 7.4 was normalized to 1.0). λex = 570 nm.

Fig. S10 SYBR Green-stained 2% agarose gel electrophores showing the self-assemble of the TDP (1 μM). Lane 1: S1; Lane 2: S1+S2; Lane 3: S1+S2+S3; Lane 4: S1+S2+S3+S4; Lane 5: S1+S2+S3+S4+R; Lane 6: S1+S2+S3+S4+R+I; Lane 7: S1+S2+S3+S4+R+I+C; and Lane 8: S1+S2+S3+S4+R+I+C+SC.

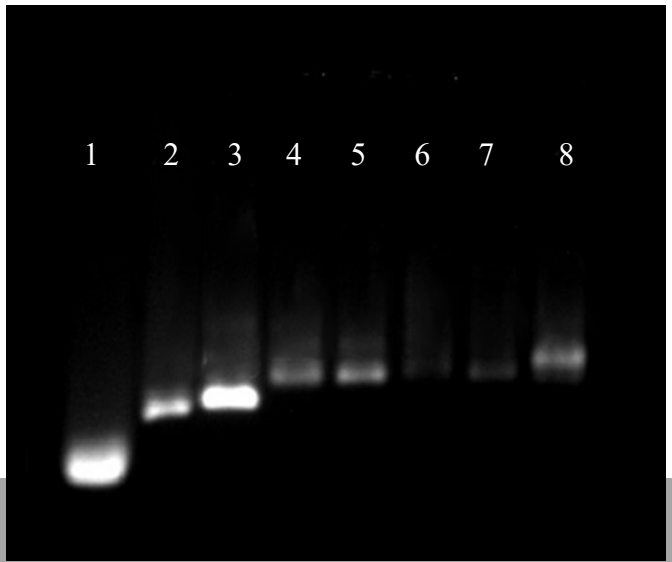

Fig. S11 SYBR Gold-stained 2% agarose gel electrophores of different DNA samples (1 μM). From lane 1 to 8: S1; S1+S2; S1+S2+S3; S1+S2+S3+S4; S1-cho+S2+S3+S4; S1-cho+S2-cho+S3+S4; S1-cho+S2-cho+S3-cho+S4; TDP.

Fig. S12. (A) Schematic diagram of tetrahedron Cy5. (B) PAGE gel assay. The TDP was incubated in fresh culture medium containing 10% FBS at 37 ℃ for different time before gel testing. λex = 635 nm.

Fig. S13. (A) Representative fluorescence emission spectra of 100 nM TDP upon incubated with different concentrations of rat liver microsomes (0, 20, 40, 60, 80, and 100 μg·mL^{-1}) and NADPH (50 μM) at 37°C for 4 h under 1% O_2. (B) Normalized Cy5 fluorescence intensity (the fluorescence intensity of Cy5 obtained upon 0 μg·mL^{-1} rat liver microsomes was normalized to 1.0) of 100 nM TDP as the functions of rat

liver microsomes ranging from 0 to 100 μg·mL^{-1}. λex = 635 nm. Error bars represent variations between three measurements.

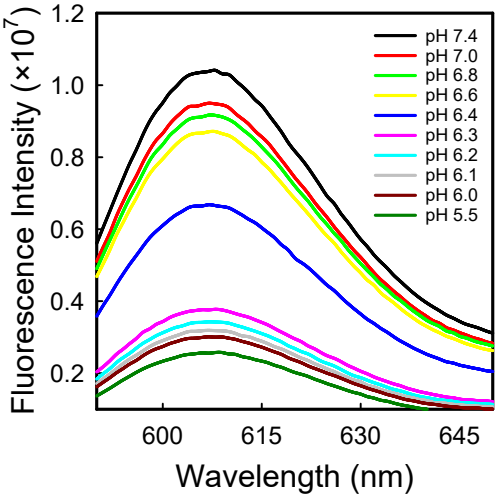

Fig. S14. (A) Representative fluorescence emission spectra of 100 nM TDP upon incubated with 0 μg·mL^{-1} rat liver microsomes and NADPH (50 μM) at 37 ℃ for 4h under 1% O_2 in buffer solution at pH 5.5-7.4. (B) Representative fluorescence emission spectra of 100 nM TDP upon incubated with 50 μg·mL^{-1} rat liver microsomes and NADPH (50 μM) at 37 ℃ for 4h under 1% O_2 in buffer solution at pH 5.5-7.4. (C) Representative fluorescence emission spectra of 100 nM TDP upon incubated with 100 μg·mL^{-1} rat liver microsomes and NADPH (50 μM) at 37 ℃ for 4h under 1% O_2 in buffer solution at pH 5.5-7.4. (D) Normalized ROX fluorescence intensity (the fluorescence intensity of ROX obtained at pH 7.4 was normalized to 1.0) of 100 nM TDP as the functions of pH after incubated with 0, 50 and 100 μg·mL^{-1} rat liver microsomes. λex = 570 nm. Error bars represent variations between three measurements.

Fig. S15. CLSM images of exosomes which incubated with the UB-TDP (ROX-red, Cy5-blue) without cholesterol modification, where exosome membranes are stained with DiO (green). Scale bars, 2 μm.

Fig. S16. (A) CLSM images of living ScaBER cells stained with DiO (green) and then incubated with UB-TDP, which was modified without (a) or with (b) cholesterol (ROX-red, Cy5-blue). Scale bars: 15 μm. (B) Flow cytometry compared the fluorescence intensity of blank (red) and TDP, without (blue) or with (yellow)

cholesterol labelling, bound to the cell membrane at 45 min. (C) Fluorescence intensity profile of white arrow regions in Fig. S16 (A(a)). (D) Fluorescence intensity profile of white arrow regions in Fig. S16 (A(b)).

Fig. S17 (A) CLSM of ScaBER cells incubated with Rho-TP (10 μM) for 2 h, and then exposed to external media at various pHs (5.5, 6.5, 7.5, and 8.5, respectively) in the presence of 10.0 μM nigericin. (B) CLSM of ScaBER cells incubated with Rho-TP (10 μM) for 2 h. (C) Intracellular pH calibration curve of Rho-TP (10 μM) in ScaBER cells. Black circles represent the mean fluorescence intensity of cells at various pHs and the black line represents the nonlinear fitting of the experimental data by SigmaPlot software. Red circles represent the mean fluorescence intensity of normal cells. Error bars represent variations between three measurements. The scale bar indicates 20 μm. λex = 559 nm. The scale bar indicates 20 μm.

Fig. S18 (A) CLSM imaging of ScaBER cells, which were incubated with 100 nM TDP (pre-treated with 0 μg·mL^{-1} rat liver microsomes) and finally incubated with PBS at pH 7.4, 7.0, 6.5, 6.0 and 5.5. (B) Normalized fluorescence intensities of ROX and plots of Cy5 acquired by ImageJ software collected from Fig S18A. For ROX: the fluorescence intensity of ROX obtained at pH 7.4 was normalized to 1.0, λex = 559 nm, λem = 570-625 nm; For Cy5: the fluorescence intensity of Cy5 obtained upon 100 μg·mL^{-1} rat liver microsomes was normalized to 1.0, λex = 640 nm, λem = 655-755 nm. Error bars represent variations between three measurements. Scale bars: 15 μm.

Fig. S19 (A) CLSM images of ScaBER cells, which were incubated with TDP that pre-treated with different concentrations of rat liver microsomes (0, 20, 50, 100 μg·mL^{-1}). (B) Normalized fluorescence intensities of ROX and Cy5 acquired by Image J software collected from Fig. S19A. (C) Normalized fluorescence intensities of ROX and plots of Cy5 acquired by Image J software collected from Fig. S19A. For ROX: the fluorescence intensity of ROX obtained at pH 7.4 was normalized to 1.0, λex = 559 nm, λem = 570-625 nm; For Cy5: the fluorescence intensity of Cy5 obtained upon 100 μg·mL^{-1} rat liver microsomes was normalized to 1.0, λex = 640 nm, λem = 655-755 nm. Error bars represent variations between three measurements. Scale bars: 15 μm.

Fig. S20 (A) CLSM imaging of ScaBER cells, which were incubated with 100 nM TDP (pre-treated with 20 μg·mL^{-1} rat liver microsomes) and finally incubated with PBS at pH 7.4, 7.0, 6.5, 6.0 and 5.5. (B) Normalized fluorescence intensities of ROX and plots of Cy5 acquired by ImageJ software collected from Fig S20A. For ROX: the fluorescence intensity of ROX obtained at pH 7.4 was normalized to 1.0, λex = 559 nm, λem = 570-625 nm; For Cy5: the fluorescence intensity of Cy5 obtained upon 100 μg·mL^{-1} rat liver microsomes was normalized to 1.0, λex = 640 nm, λem = 655-755 nm. Error bars represent variations between three measurements. Scale bars: 15 μm.

Fig. S21 (A) CLSM imaging of ScaBER cells, which were incubated with 100 nM TDP (pre-treated with 50 μg·mL^{-1} rat liver microsomes) and finally incubated with PBS at pH 7.4, 7.0, 6.5, 6.0 and 5.5. (B) Normalized fluorescence intensities of ROX and plots of Cy5 acquired by ImageJ software collected from Fig S21A. For ROX: the fluorescence intensity of ROX obtained at pH 7.4 was normalized to 1.0, λex = 559 nm, λem = 570-625 nm; For Cy5: the fluorescence intensity of Cy5 obtained upon 100 μg·mL^{-1} rat liver microsomes was normalized to 1.0, λex = 640 nm, λem = 655-755 nm. Error bars represent variations between three measurements. Scale bars: 15 μm.

Fig. S22 (A) CLSM imaging of ScaBER cells, which were incubated with 100 nM TDP (pre-treated with 100 μg·mL^{-1} rat liver microsomes) and finally incubated with PBS at pH 7.4, 7.0, 6.5, 6.0 and 5.5. (B) Normalized fluorescence intensities of ROX and plots of Cy5 acquired by ImageJ software collected from Fig S22A. For ROX: the fluorescence intensity of ROX obtained at pH 7.4 was normalized to 1.0, λex = 559 nm, λem = 570-625 nm; For Cy5: the fluorescence intensity of Cy5 obtained upon

100 μg·mL^{-1} rat liver microsomes was normalized to 1.0, λex = 640 nm, λem = 655-755 nm. Error bars represent variations between three measurements. Scale bars: 15 μm.

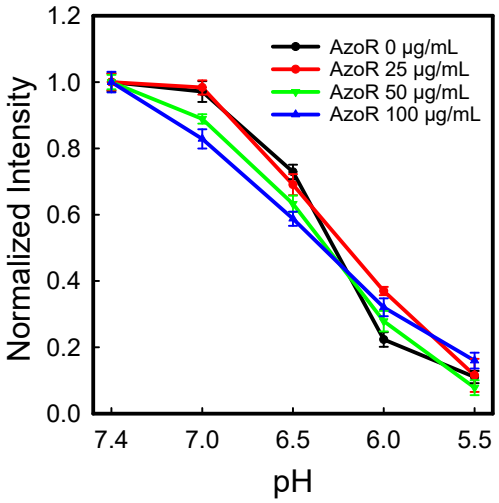

Fig. S23 Normalized fluorescence intensity of ROX collected from Fig. S18, 20-22 as the function of pH upon incubated with 0, 20, 50 and 100 μg·mL-1 rat liver microsomes.

Fig. S24 (A) CLSM of ScaBER cells incubated with Rho-TP (10 μM) for 2 h, and then exposed to PBS at various pH (5.5, 6.5, 7.5, and 8.5, respectively). (B) The normalized intensity of Fig. S21 was quantified according to Fig. S24 by ImageJ software. The scale bar indicates 20 μm. λex = 559 nm. The scale bar indicates 20 μm

Fig. S25 (A) Representative fluorescence emission spectra of TDPE upon incubated with different concentrations of rat liver microsomes (0, 20, 40, 60, 80, and 100 $\mu g \cdot mL^{-1}$) and NADPH (50 μM) at 37°C for 4 h under 1% O_2. (B) Normalized Cy5 fluorescence intensity (the fluorescence intensity of Cy5 obtained upon 0 $\mu g \cdot mL^{-1}$ rat liver microsomes was normalized to 1.0) of TDPE as the functions of rat liver microsomes ranging from 0 to 100 $\mu g \cdot mL^{-1}$. λex = 635 nm. Error bars represent variations between three measurements.

Fig. S26 (A) Representative fluorescence emission spectra of the TDPE upon incubated with 0 $\mu g \cdot mL^{-1}$ rat liver microsomes and NADPH (50 μM) at 37 ℃ for 4h under 1% O_2 in buffer solution at pH 5.5-7.4. (B) Representative fluorescence emission spectra of the TDPE upon incubated with 50 $\mu g \cdot mL^{-1}$ rat liver microsomes and NADPH (50 μM) at 37 ℃ for 4h under 1% O_2 in buffer solution at pH 5.5-7.4. (C) Representative fluorescence emission spectra of the TDPE upon incubated with 100 $\mu g \cdot mL^{-1}$ rat liver microsomes and NADPH (50 μM) at 37 ℃ for 4h under 1% O_2 in buffer solution at pH 5.5-7.4. (D) Normalized ROX fluorescence intensity (the fluorescence intensity of ROX obtained at pH 7.4 was normalized to 1.0) of the TDPE as the functions of pH after incubated with 0, 50 and 100 $\mu g \cdot mL^{-1}$ rat liver

microsomes. λex = 570 nm. Error bars represent variations between three measurements.

Fig. S27 (A) Time-dependent in vivo fluorescence imaging of mice that were injected with TDPE (DNA probe dose of 1 μmol/kg). n = 3. Circles *I* represent normal regions, circles *II* represent simulated inflammation regions by LPS, circles *III* represent simulated hypoxic regions after exercise by $CoCl_2$, and Circles *IV* represent tumor regions. All injections were employed in four regions. For ROX: λex = 560 nm; For Cy5: λex = 620 nm. (B) Corresponding mean fluorescence intensity of ROX from Fig. S27 (A(a)). (C) Corresponding mean fluorescence intensity of Cy5 from Fig. S27 (A (b)). Error bars represent variations between three measurements.

Fig. S28 (A) Time-dependent in vivo fluorescence imaging of mice that were injected with nanospring (a) TDP (b) and TDPE (c) (DNA probe dose of 1 μmol/kg). n = 3. λex = 560 nm. (B) Corresponding mean fluorescence intensity of ROX from Fig. S28 (A). Error bars represent variations between three measurements.

References:

[1] S. Chen, M. Chen, J. Yang, X. Zeng, Y. Zhou, S. Yang, R. Yang, Q. Yuan and J. Zheng, *Small,* 2021, 17, 2100243.

[2] W. Shi, X. Li and H. Ma, *Angew Chem Int Edit*., 2012, 51, 6432.