Electronic Supplementary Material (ESI) for Chemical Science.
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Supporting information

Materials

1,3,5-Triformylbenzene, 1,4-diaminobenzene and polyvinylpyrrolidone (PVP-K30) were obtained from Maklin. Acetic acid and acetonitrile were purchased from BeiJing chemical works. Polyethyleneimine (PEI, Mw = 1800Da), SH−PEG-3000−OCH_3 and sodium borohydride ($NaBH_4$) were purchased from Aladdin. Saccharomyces cerevisiae (Baker's yeast) was obtained from Thermo Fisher Scientific. Hydrogen tetrachloroaurate (III) ($HAuCl_4•3H_2O$) was purchased from Sinopharm Chemical Reagent Co. (Shanghai, China). All chemicals were used as received without further purification. The Milli-Q water was obtained from the Milli-Q System.

The preparation of artificial probiotics

Firstly, 1,3,5-Triformylbenzene (12.5 mg) and 1,4-diaminobenzene (15 mg) were dissolved in 10 mL of acetonitrile in a 16 mL capped vial, and then, acetic acid (0.05 mL) was added. When the solution turned yellow, polyvinylpyrrolidone-40000 (0.1 g mL^{-1} in acetonitrile) was added, and the mixture was stirred at room temperature for 24 h.

Then, the as-synthesized COF (10 mg) were mixed with SH−PEG-3000−OCH_3 (5 mg) in 4 mL of deionized water. After stirring for 1 h, the $HAuCl_4$ solution was added into the mixture (34 μL, 10% w/v) for 15 min. A freshly prepared $NaBH_4$ (50μL; 0.1m M) was added into the above aqueous solution under vigorous stirring. After mixture, the resulting suspension was stirred for 5 min.

Finally, to encapsulate the COF@Au nanoparticles in YS, 5 mg YS was pre-incubated

with 2.5 mg PEI in 1 ml CBS. After thorough rinsing with deionized water, PEI-treated YS was incubated with COF@Au under vigorous stirring for 1h. The obtained Aprobiotis were washed 3 times with Milli-Q water and collected by centrifuging at 3,000 rpm. for 5 min.

Glucose oxidase-like activity of Aprobiotics

The reaction path of Au NPs catalysis is the same as that of natural glucose oxidase, except that OH^- is used as a Brønsted base to abstract H^+ from glucose. The use of OH^- as a Brønsted base corresponds to that glucose oxidation and alcohol oxidation are faster under alkaline conditions. Briefly, Aprobiotics (100 μg mL^{-1}) and GOx (with the same concentration) were mixed with glucose (10 mM mL^{-1}) in PBS (pH 7.4), respectively. The reaction product, gluconic acid, was assayed by the reaction with hydroxylamine and subsequent complex with $FeCl_3$, which led to a red complex with a major absorbance at 505 nm. Firstly, 250 μL of solution 1 (5 mM EDTA and 0.15 mM triethylamine in water) and 25 μL of solution 2 (3 M NH_2OH in water) were added to the catalytic reaction solution. After 25 min of incubation, 125 μL of solution 3 (1 M HCl, 0.1 M $FeCl_3$, and 0.25 M CCl_3COOH in water) was added to the above aqueous solution, and the reaction was allowed to proceed for 5 min before spectral measurements.

Furthermore, the dissolved O_2 and PH in Aprobiotics (200 μg mL^{-1}) solution was also measured in the absence or presence of glucose (1 mg mL^{-1}) using a portable dissolved oxygen meter and pH meter, respectively (PH=7.4, 0.5 mM PBS).

The apparent kinetic parameters were calculated from the Lineweavere-Burk plot: 1/V

= (K_m/V_{max}) *(1/C) + $1/V_{max}$, where v is the initial velocity, Vmax is the maximal reaction velocity, and C is the concentration of substrate. The rate of the catalytic reaction was defined as the amount of generated product, gluconic acid, in a fixed time interval of 30 min at 37 ℃. The amount of gluconic acid was determined by continuous titration with NaOH. In detail, Aprobiotics (AuNPs: 1.25 μM.L^{-1}) was incubated with a series of concentrations of glucose for 30 min, respectively. Next, the obtained catalytic product gluconic acid was titrated with 0.1 M of NaOH with a pH indicator, phenolphthalein that shows sharp colorless-tored change at pH 8.2.

The superoxide radicals scavenging activity of Aprobiotics

The Superoxide radicals scavenging activity of Aprobiotics by measuring inhibition of the photoreduction of nitro blue tetrazolium (NBT). The solutions containing riboflavin (20 μM), methionine (0.013 M), NBT (75μM), and composites of various concentrations were prepared with 25 mM pH 7.4 phosphate buffer. The mixtures were illuminated by a lamp with a constant light intensity for 3 min at room temperature. After illumination, immediately the absorbance was measured at 560 nm.

Hydroxyl radicals scavenging activity of Aprbiotics

The Hydroxyl radicals scavenging activity of COF, COF@Au, YS, Aprbiotics were assayed by measuring inhibition of the generation of 2-hydroxyterephthalic acid. The solutions containing terephthalic acid (TA) (0.5 mM), H_2O_2 (10 mM) and nanocomposites of various concentrations were prepared with 25 mM (pH 7.4) phosphate buffer. The mixtures were incubated for 24 h. TA is a non-fluorescent compound which can capture OH• to produce the highly fluorescent 2-

hydroxyterephthalic acid with an emission peak at 435 nm upon excitation wavelength of 315 nm.

$ABTS^{+}$• scavenging activity of Apribiotic

Firstly, $ABTS^{+}$• (7 mM) was dissolved in water containing $K_2S_2O_8$ (2.45 mM) to produce $ABTS^{+}$•, and above mixture was kept in dark at room temperature for 24 h before use, which was named as stock solution. Secondly, the concentration of $ABTS^{+}$• of above stock solution was adjusted to 34.5 μM with PBS (10 mM, pH 7.4). After the addition of Aprobiotics, above solutions were further incubated in dark for another 30 min. Thirdly, UV-Vis-NIR absorption spectra of above diluted solutions of $ABTS^{+}$• were monitored, and the absorbance at 734 nm was recorded. $ABTS^{+}$• scavenging efficacy was calculated by using the ratio of neutralized $ABTS^{+}$• to the overall $ABTS^{+}$•.

• NO scavenging activity of Aprbiotics

The ·NO scavenging activities of Aprobiotics were tested by measuring the inhibition of the generation of nitrite. Nitric oxide was generated by Sodium Nitroferricyanide (III) Dihydrate (SNP) in an aqueous solution at physiological pH and was then detected by the Griess reagent after reaction with dissolved oxygen to form nitrite. Scavengers of ·NO compete with oxygen, leading to reduced production of NO_2^-. 100 μL PBS (pH 7.4) of SNP (10 mM) and Aprobiotics (0-100 μg/mL) were incubated for 180 min at 25℃. Then, the mixtures were centrifuged at 12 000 rpm for 10 min, and the concentration of NO_2^- in the supernatant was subsequently determined by Nitric Oxide Assay Kit and the OD_{540} was recorded by SpectraMax M2 Molecular Devices.

Cell culture

The RAW264.7 and CT26 cells were supplied by American Type Culture Collection (ATCC). The cells were cultured in Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum and 1% Penicillin-Streptomycin at 37 °C in a humidified atmosphere of 5% CO_2.

In vitro cytotoxicity measurement

RAW264.7 and CT26 cells were seeded in 96-well plates with a density of 5000 cells per well and cultured overnight. Aprobiotics with different concentrations was added to the wells. After cultured for 24 h the medium was removed, following by washing with PBS. 100 μL of 10% MTT solution was added to the above each well, and then the plate were incubated for 4 h at 37 °C. A microplate reader was used to measure the absorbance at 490 nm.

Cellular uptake behaviors of Aprobiotics

RAW264.7 and CT26 cells was randomly planted in 24-well plates and allowed to adhere for 24 h. RB-labeled Aprobiotics was added and incubated with the cells for 4 h. And then, the cells were washed by PBS for 3 times before fluorescence analysis.

Determination of ROS scavenging activity in vitro

For ROS detection, RAW264.7 and CT26 cells were randomly seeded in 6-well plates in DMEM for 24 h before further manipulation. Then cells were treated by Aprobiotics for 4 h. Afterward, H_2O_2 was added and incubated for 1 h. The treated cells were washed with PBS twice and incubated with 10 μM of DCFH-DA for 30 min. After removing the unloaded probe with PBS, the fluorescence intensity of cells

was monitored by confocal laser scanning microscope (CLSM).

Immunomodulatory experiments in vitro

Briefly, RAW264.7 cells were co-incubated with Aprobiotics in 24-well plates for 4 h. Afterward, H2O2 was added and incubated for 2 day, the supernatant was removed, while the RAW264.7 cells were fixed with paraformaldehyde (4%), permeabilized using Triton-X (0.1%), and blocked by bovine serum albumin (1%, BSA). After washing, the cells were incubated with primary monoclonal antibodies for FITC-CD206 (1:800, biolend) and PE-CD86 (1:100, Abcam) for 24 h at 4 °C. Subsequently, the nuclei were stained by DAPI. Finally, the cells were observed and imaged with CLSM.

RT-qPCR assay

The RAW264.7 cells treated with either H_2O_2 or Aprobiotics for 1 day, they were lysed. After extraction of total RNA, the PrimeScript RT Reagent Kit (Beyotime, china) was used to synthesize the complementary DNA (cDNA). Subsequently, the SYBR Premix Ex TaqII Kit and PCR instrument were used to detect the expression levels of iNOS and TNF-α (pro-inflammatory) and Arg-1 and IL-10 (anti-inflammatory) with three technical repeats for each sample. In this section, the GAPDH gene served as the housekeeping gene, and the gene expression levels in each group were evaluated using the $2^{-\Delta\Delta Ct}$ method. All primers data were listed in Table S1.

Biocompatibility evaluation

Kunming mice (n = 5) were divided into 2 groups at random, containing PBS

(control), Aprobiotics 20 mg kg^{-1}). After7 and 28 days, the mice were sacrificed and the blood was used to carry out serum biochemistry assay and complete blood panel analysis at the Changchun Chain Medical Laboratories, Inc. The major organs containing kidney, lung, liver, spleen and heart were collected to stain with H&E.

DSS-induced model of colitis

Six-week-old female kunming mice were housed in groups of five mice per cage and acclimatized for 1week before inclusion in the study. Mice received 2% DSS (40kDa; Maklin) supplemented in the drinking water for 5, followed by normal water. Control healthy mice were provided with normal water only. Then 20mg kg^{-1} of COF, COF@Au, Aprobiotics or PBS was administered via an oral route into mice on predetermined days. Changes in body weight were assessed daily over the 5-12 days experimental period. Faeces were collected on the predetermined day for microbiome analysis. On the last day of the experiment, mice were sacrificed and the entire colon was excised. Colon length was measured and gently washed with physiological saline. Then, two pieces 0.5cm in length of the distal section were used for histological assessment and immunofluorescence staining. The remaining colon tissue samples were used for histological analyses.

Histology

For histological analyses, Harvested mouse tissues were fixed with paraformaldehyde (10%) after washing with 0.9% NaCl solution, and dehydrated. Subsequently, organ samples were embedded in paraffin, sectioned, stained with hematoxylin and eosin (H&E). Then, stained slides were observed on an Olympus BX-51 optical system.

Immunohistochemistry experiment

For immunoflourescent staining, 5 μm thickness paraffin sections were deparaffinized, washed three times in PBS for 5 min, and blocked with serum (5%) for 30 min. After sections' fixation in acetone at -20 °C, the sections were incubated with primary antibodies against IL-6 (1:200, Bioss Antibodies) or TNF-alpha (TNF-α, 1:200, Bioss Antibodies) at 4 °C overnight. Then the mixture was incubated with secondary antibodies (1:200, Bioss Antibodies) for 2 h at room temperature. Immunofluorescence images were acquired with an Olympus BX-51 optical system.

Microbiome analysis

a) DNA extraction

Total community genomic DNA extraction was performed using a E.Z.N.A.Soil DNA Kit (Omega, USA), following the manufacturer's instructions. We measured the concentration of the DNA using a Qubit 2.0(life,USA) to ensure that adequate amounts of high-quality genomic DNA had been extracted .

b) 16S rRNA gene amplification by PCR

Our target was the V3–V4 hypervariable region of the bacterial 16S rRNA gene. PCR was started immediately after the DNA was extracted.The 16S rRNA V3–V4 amplicon was amplified using KAPA HiFi Hot Start Ready Mix (2×) (TaKaRa Bio Inc., Japan). Two universal bacterial 16S rRNA gene amplicon PCR primers (PAGE purified) were used: the amplicon PCR forward primer (CCTACGGGNGGCWGCAG) and amplicon PCR reverse primer (GACTACHVGGGTATCTAATCC). The reaction was set up as follows: microbial

DNA (10 ng μl^{-1}) 2 µl; amplicon PCR forward primer (10 µM) 1; amplicon PCR reverse primer (10 µM) 1 µl; 2X KAPA HiFi Hot Start Ready Mix 15 µl (total 30 µl). The plate was sealed and PCR performed in a thermal instrument (Applied Biosystems 9700, USA) using the following program: 1 cycle of denaturing at 95 ° C for 3 min, first 5 cycles of denaturing at 95° C for 30 s, annealing at 45° C for 30 s, elongation at 72° C for 30 s, then 20 cycles of denaturing at 95° C for 30 s, annealing at 55° C for 30 s, elongation at 72° C for 30 s and a final extension at 72° C for 5 min. The PCR products were checked using electrophoresis in 1 % (w/v) agarose gels in TBE buffer (Tris, boric acid, EDTA) stained with ethidium bromide (EB) and visualized under UV light.

c) 16S gene library construction,quantification,and sequencing

We used AMPure XP beads to purify the free primers and primer dimer species in the amplicon product.Samples were delivered to Sangon BioTech (shanghai) for library construction using universal Illumina adaptor and index. Before sequencing, the DNA concentration of each PCR product was determined using a Qubit® 2.0 Green double-stranded DNA assay and it was quality controlled using a bioanalyzer (Agilent 2100, USA). Depending on coverage needs, all libraries can be pooled for one run.The amplicons from each reaction mixture were pooled in equimolar ratios based on their concentration. Sequencing was performed using the Illumina MiSeq system (Illumina MiSeq, USA), according to the manufacturer's instructions.

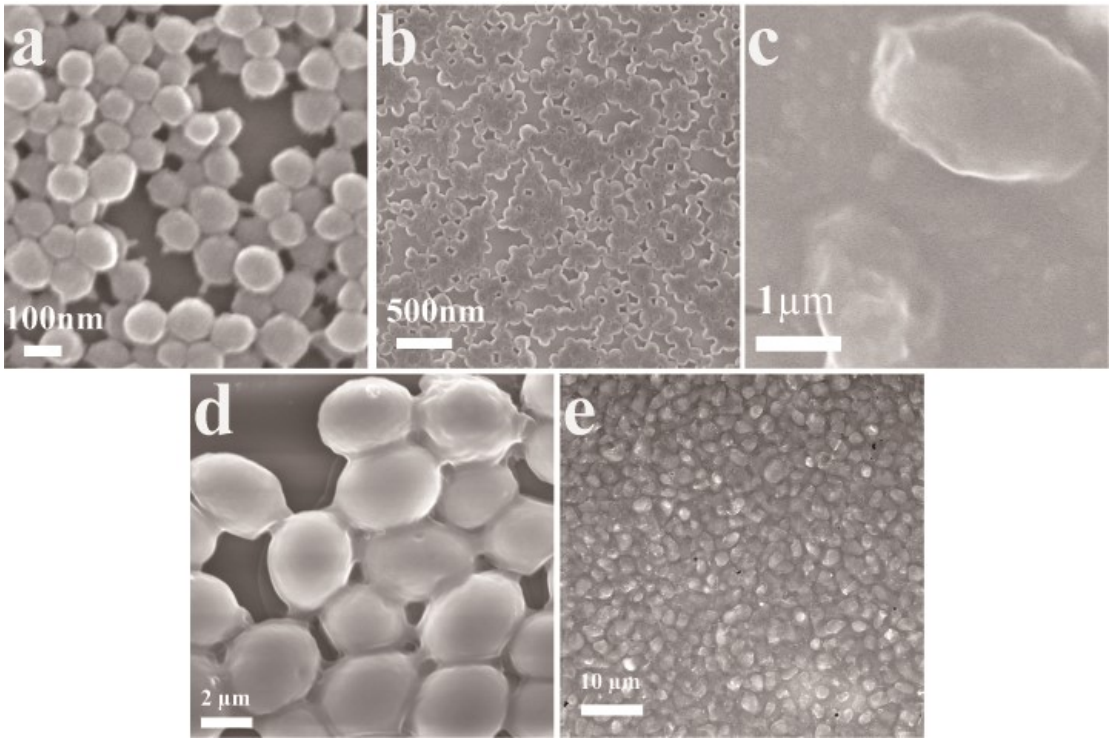

Figure S1 SEM images of (a) COF (b) COF@Au (c) Aprobiotics (d) yeast and (e) YS.

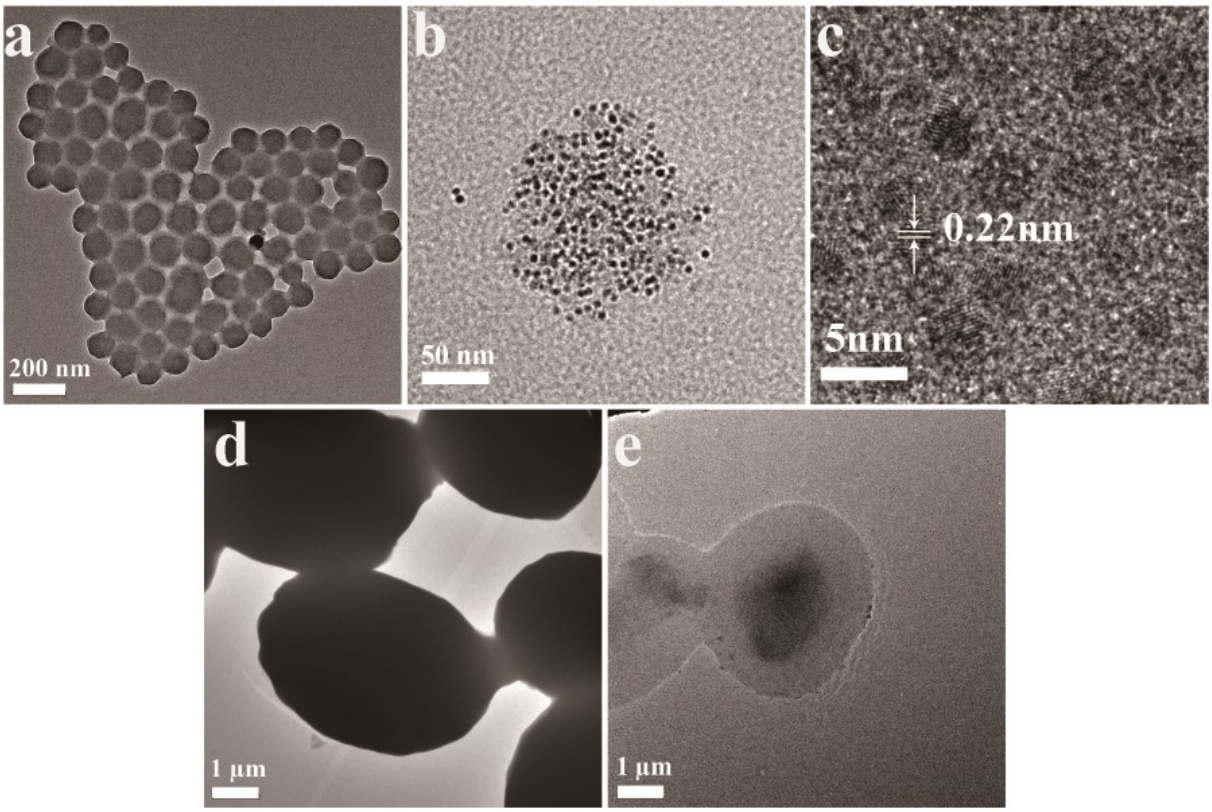

Figure S2 TEM images of (a) COF (b) COF@Au (c) Au nanoparticles (d) yeast (e) YS.

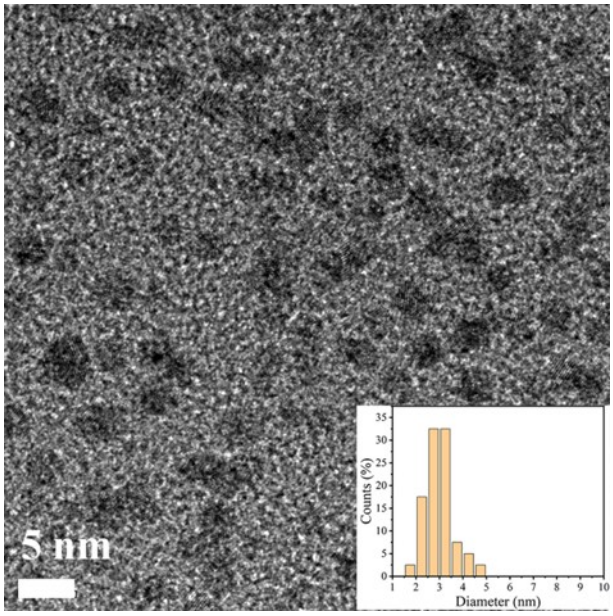

Figure S3 TEM image of ultrasmall Au nanoparticles in COF@Au.

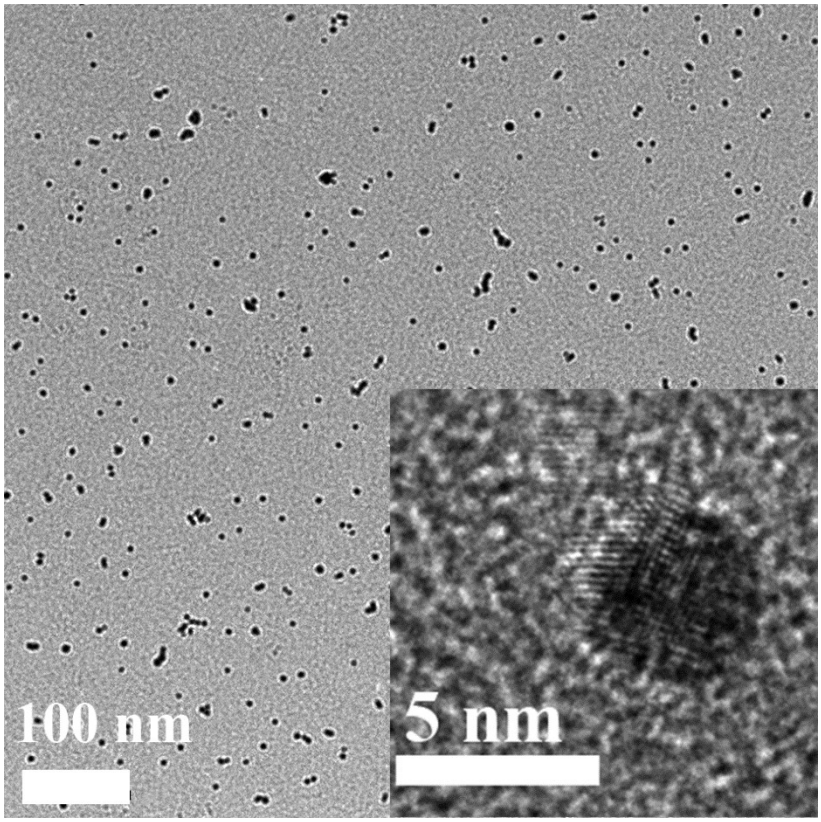

Figure S4 TEM image of ultrasmall Au nanoparticles.

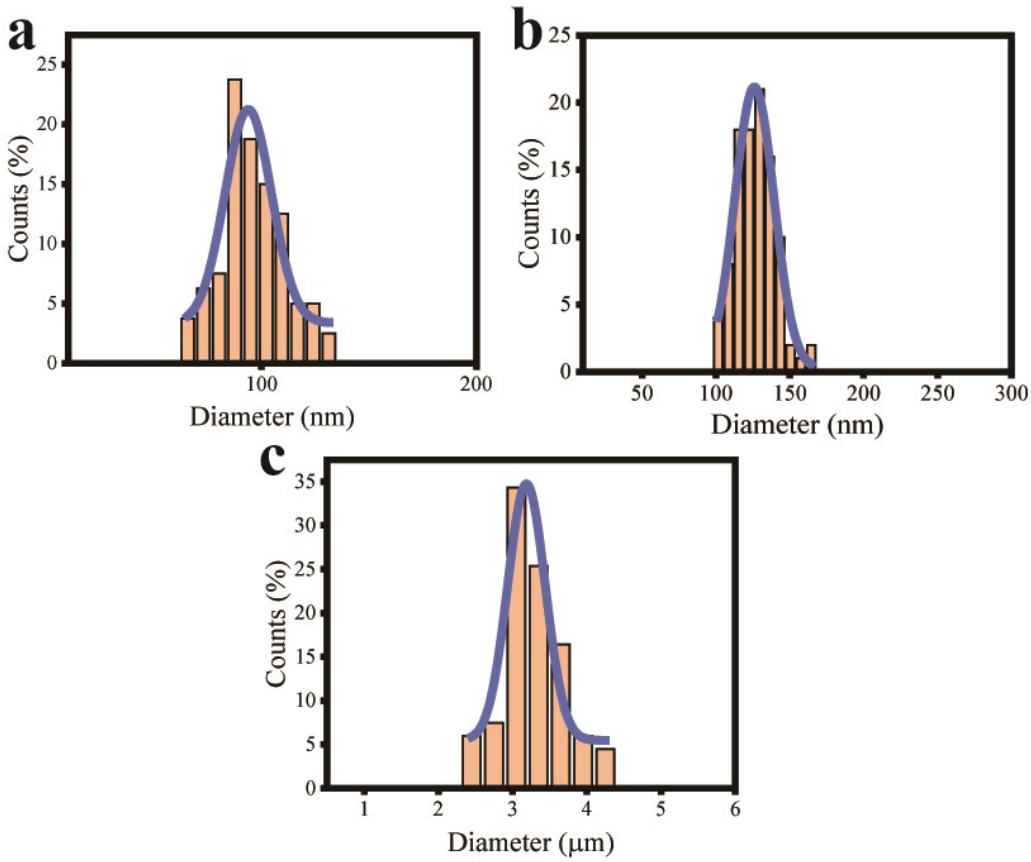

Figure S5 Size distribution of (a) COF (b) COF@Au (c) YS.

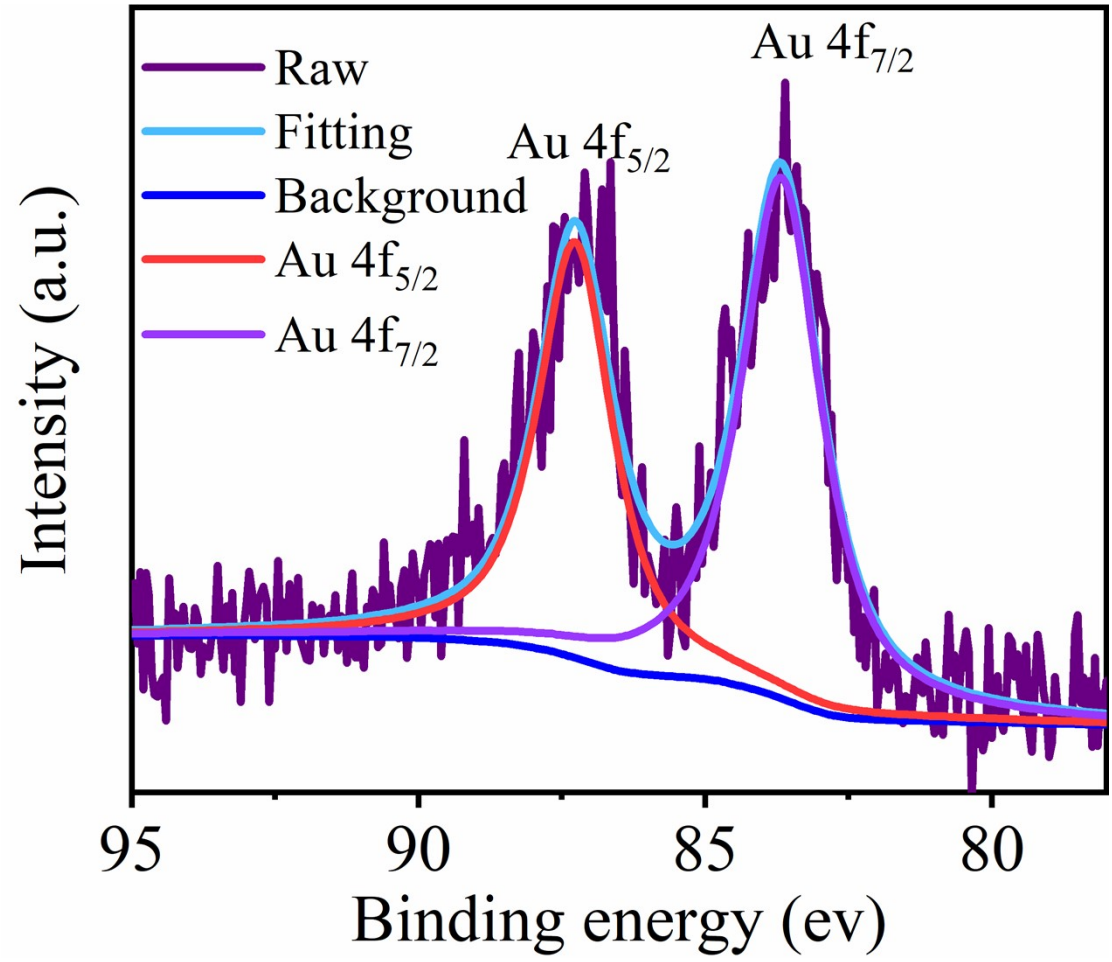

Figure S6 XPS spectra of Au 4f for Aprobiotics.

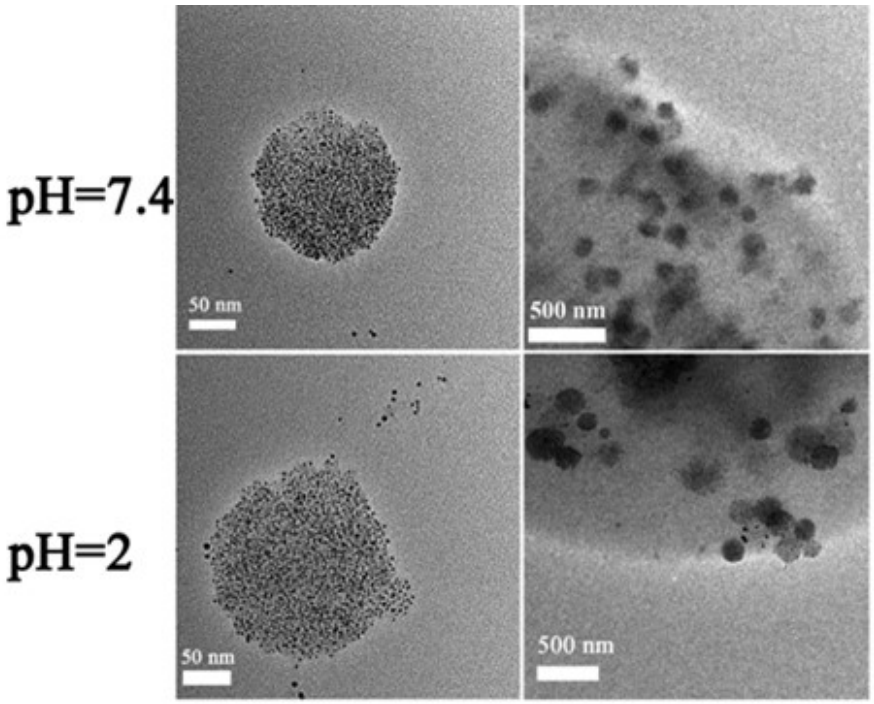

Figure S7 The stability of Aprobiotics within both physiological conditions and the acidic stomach conditions.

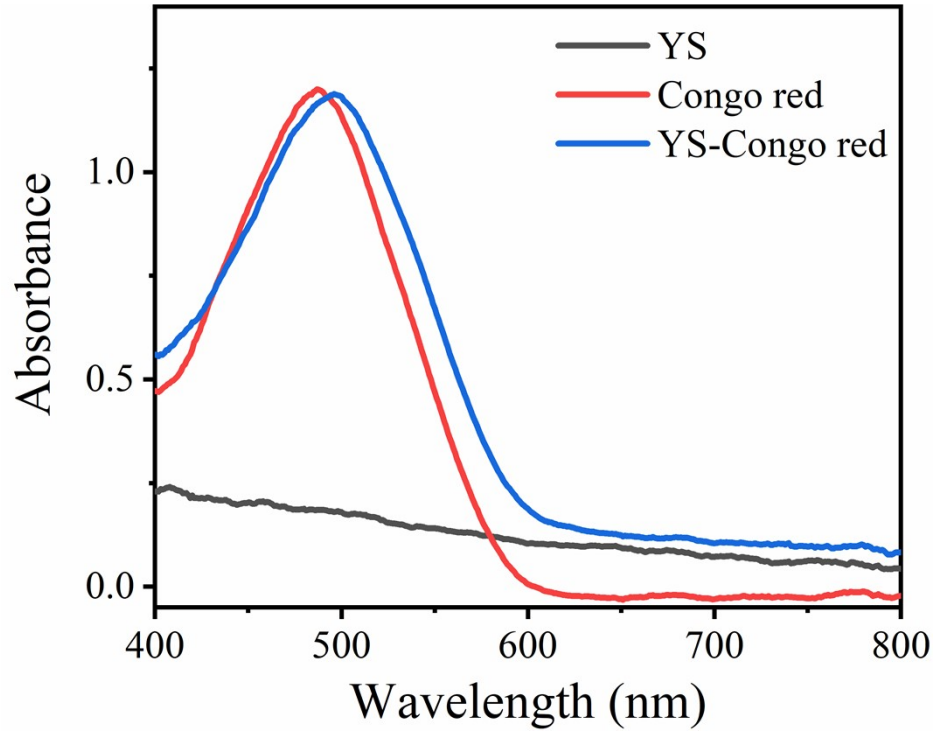

Figure S8 The UV-Vis spectra of YS, congo red, and YS-congo red.

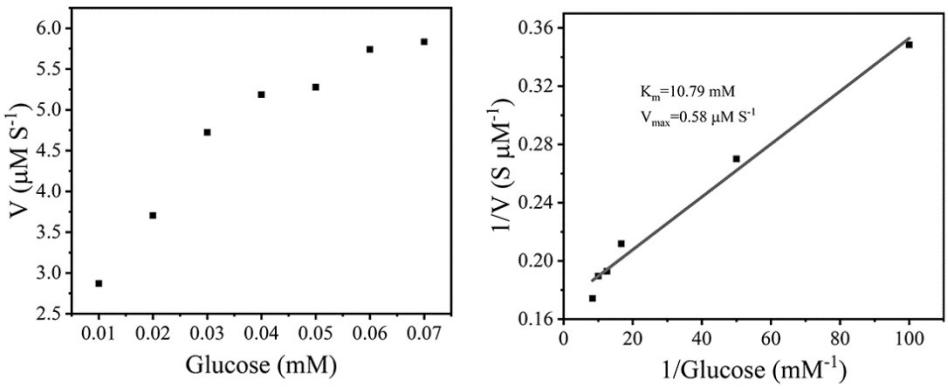

Figure S9 Lineweaver–Burk plot of the reciprocals of initial rate vs. substrate concentration for the determination of kinetic parameters Km and Vmax of Aprobiotics.

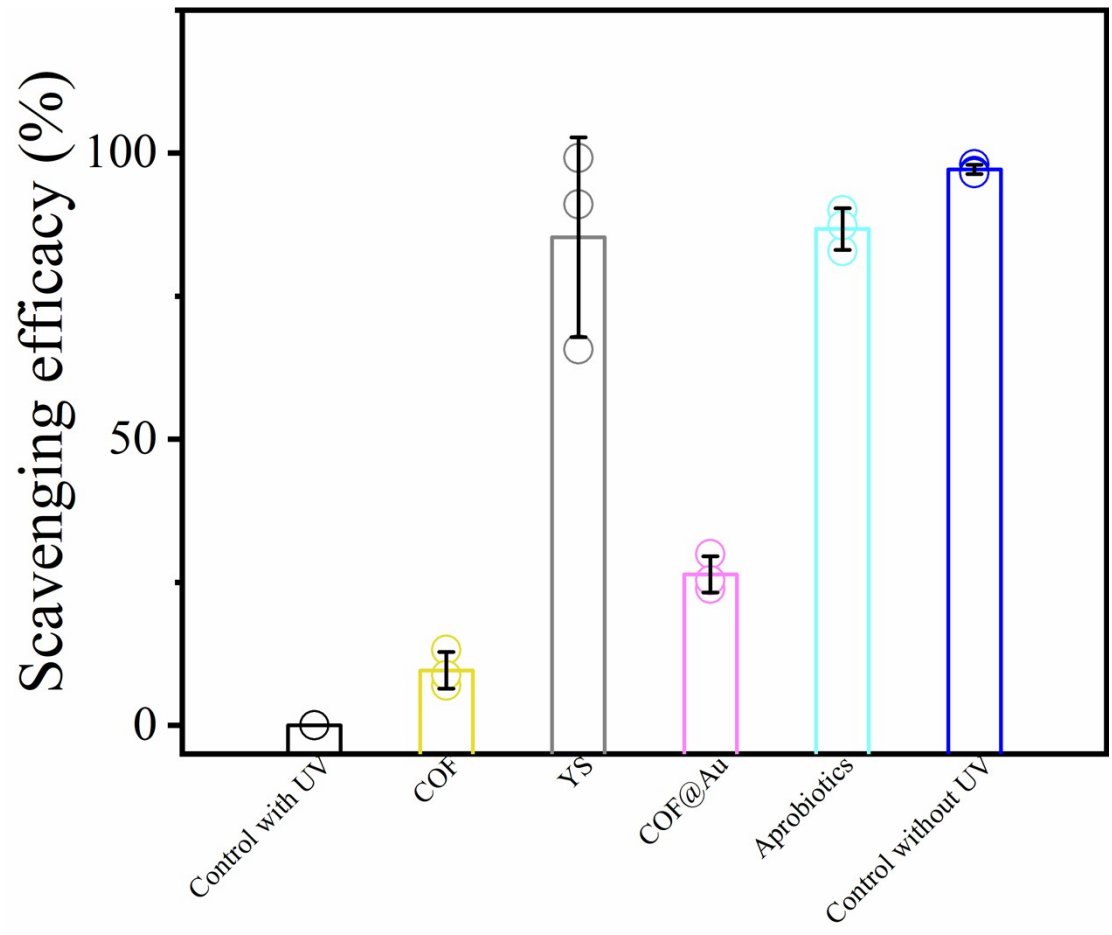

Figure S10 The $O_2^{-\bullet}$scavenging activities of COF, COF@Au, YS and Aprobiotics.

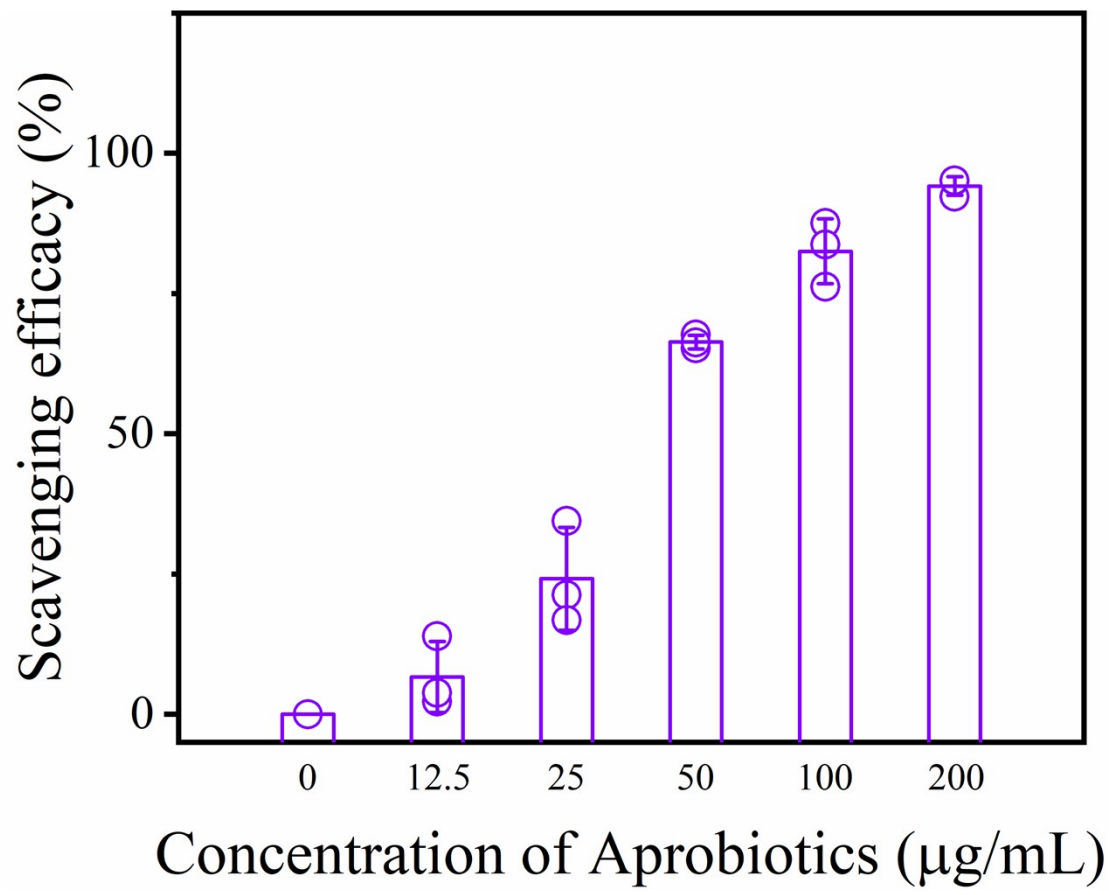

Figure S11 The $O_2^{-\bullet}$scavenging efficacies of different concentration of Aprobiotics.

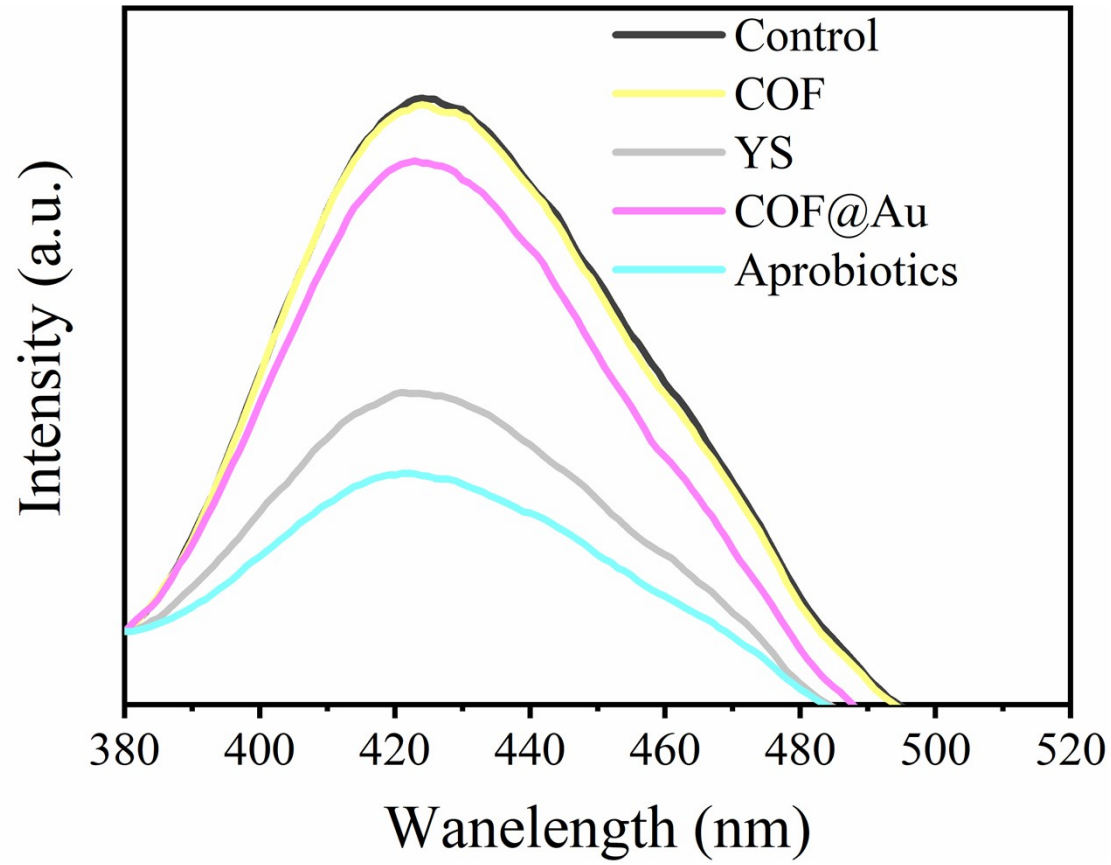

Figure S12 OH• scavenging activities of COF, COF@Au, YS and Aprobiotics.

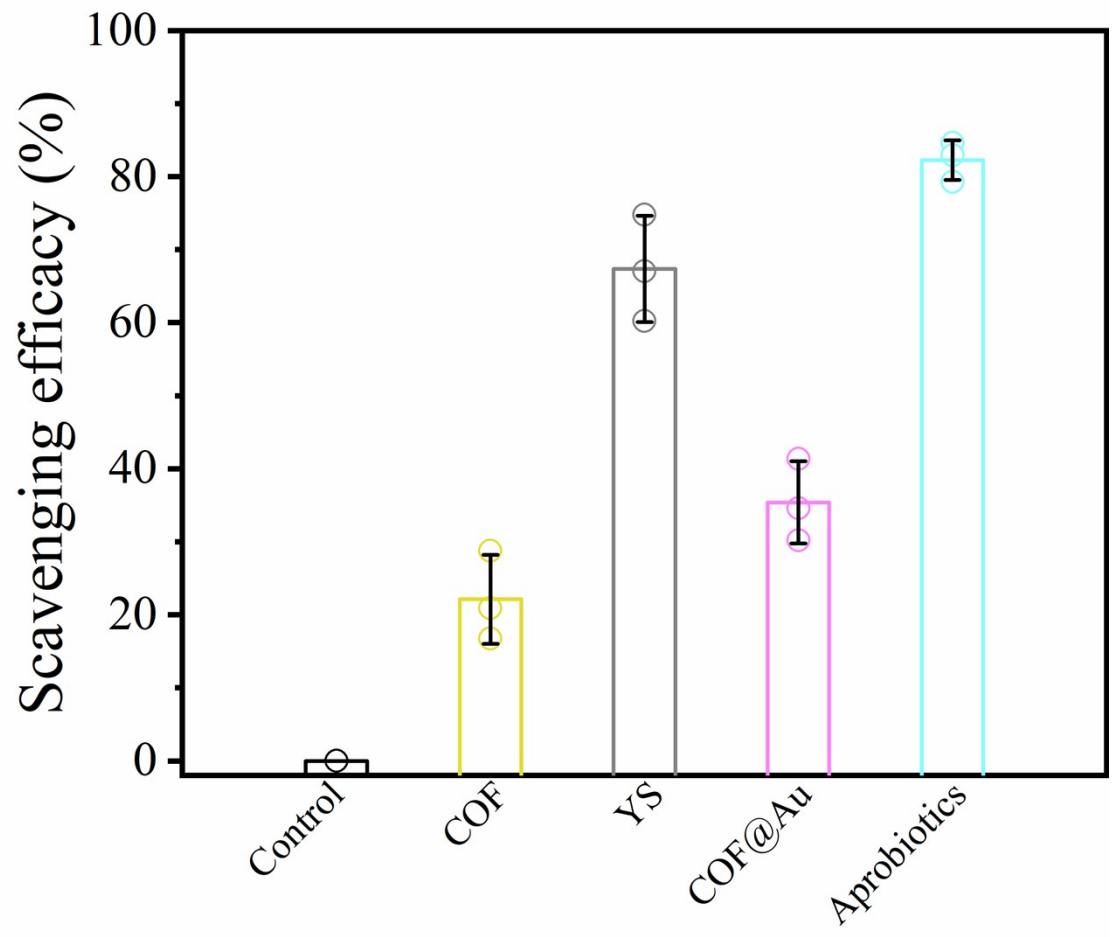

Figure S13 $ABTS^{+\bullet}$ scavenging efficacies of COF, YS, COF@Au, Aprobiotics.

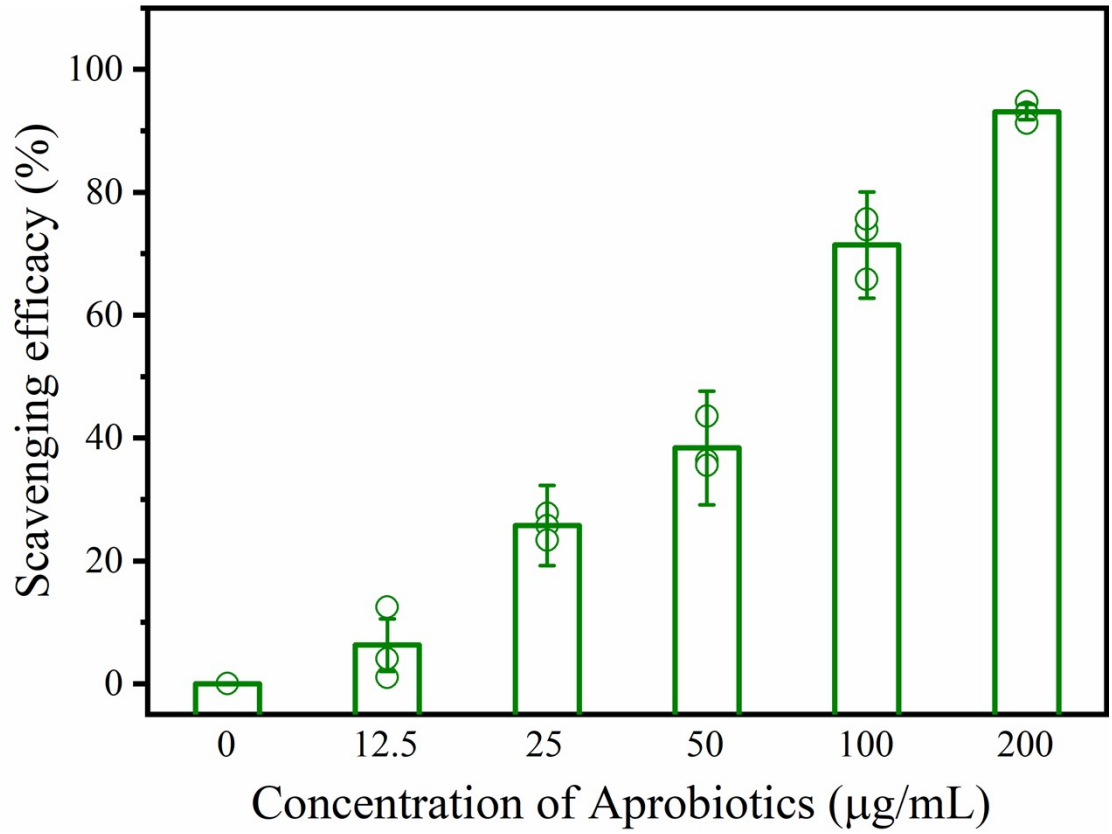

Figure S14 $ABTS^{+}$• scavenging efficacies of different concentration of Aprobiotics.

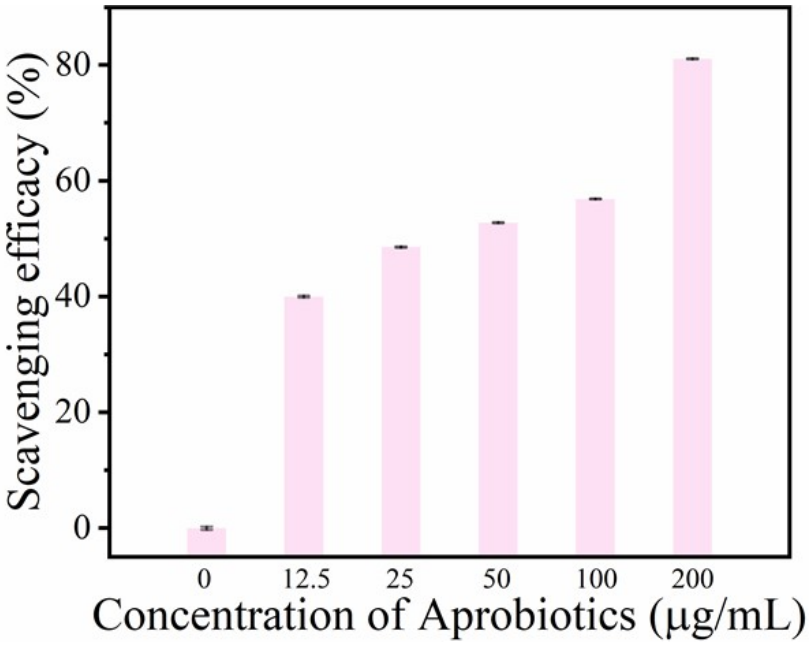

Figure S15 The NO scavenging efficacies of different concentration of Aprobiotics.

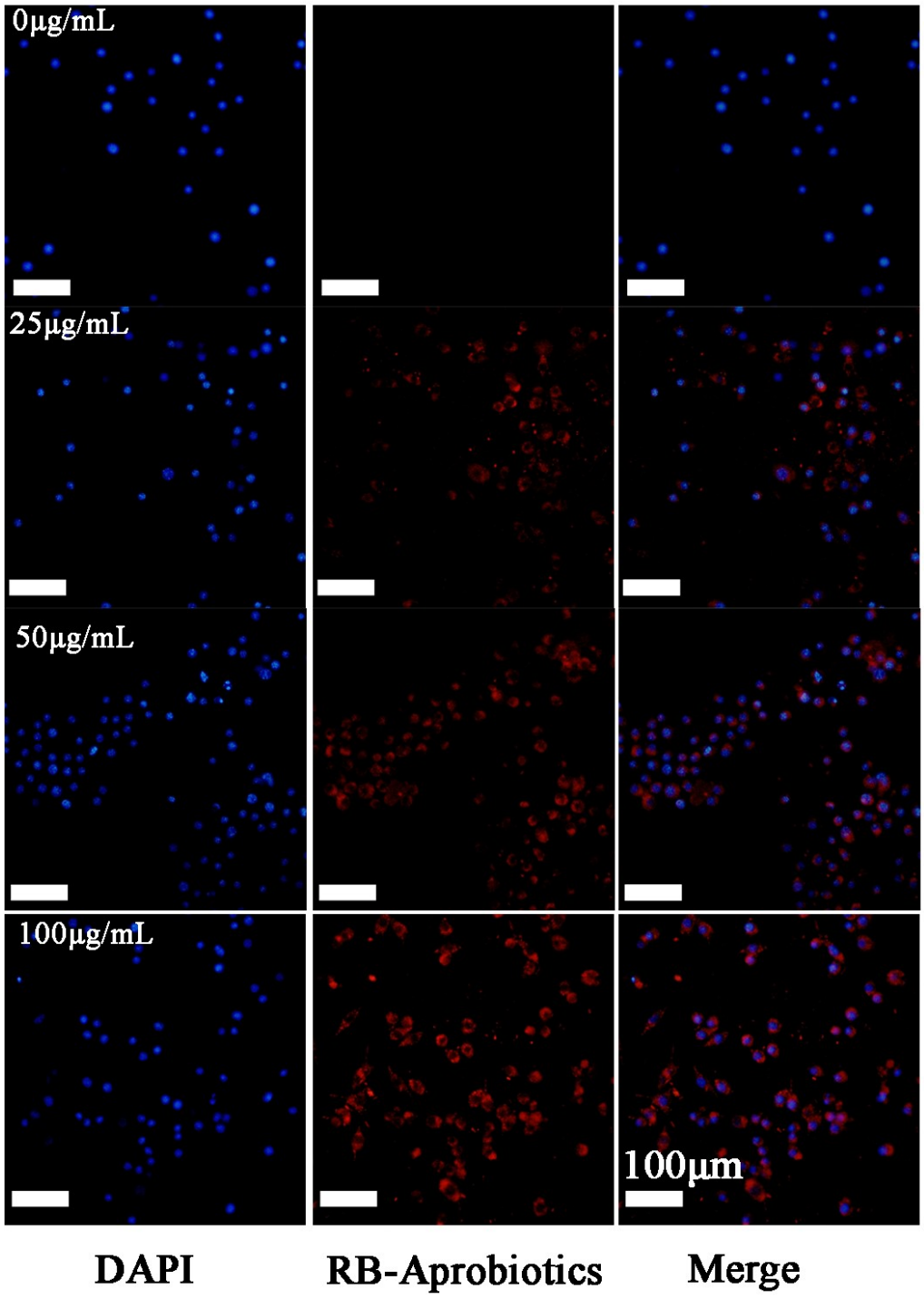

Figure S16 LSCM images of macrophages after incubation with different concentration of RB-Aprobiotics at 37 °C for 4 h.

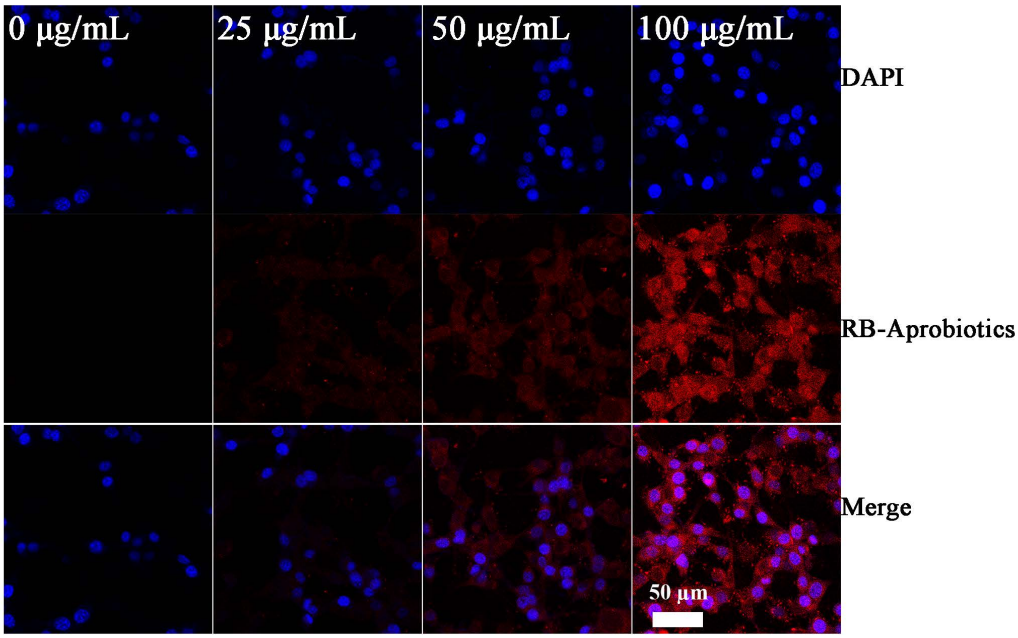

Figure S17 LSCM images of CT26 after incubation with different concentration of RB-Aprobiotics at 37 °C for 4 h.

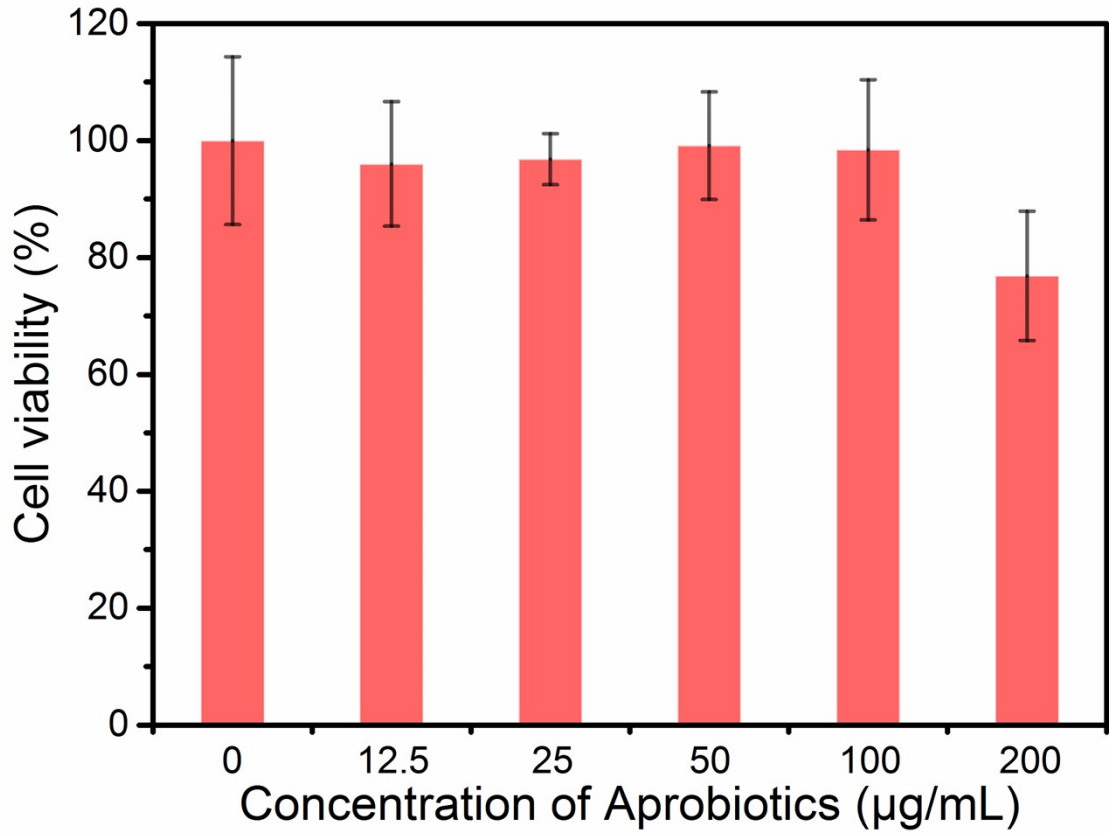

Figure S18 Effect of variable concentrations of Aprobiotics on viability of RAW264.7 cells.

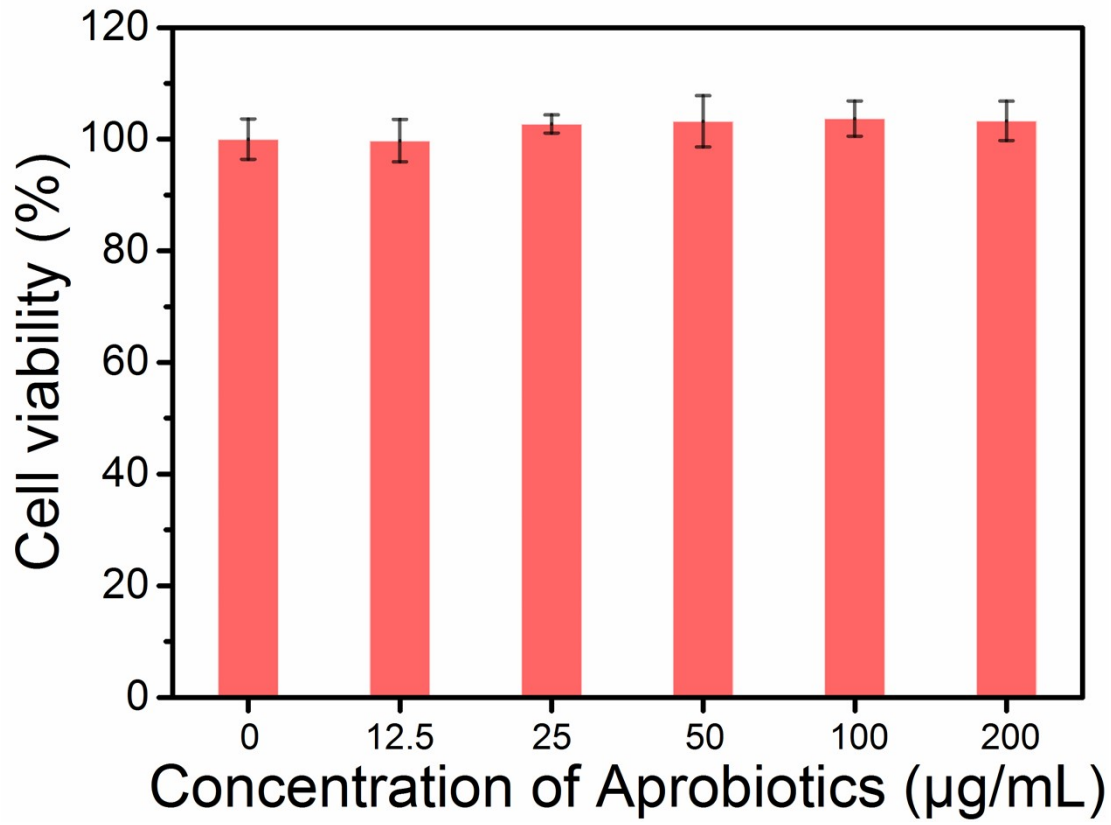

Figure S19 Effect of variable concentrations of Aprobiotics on viability of CT26 cells.

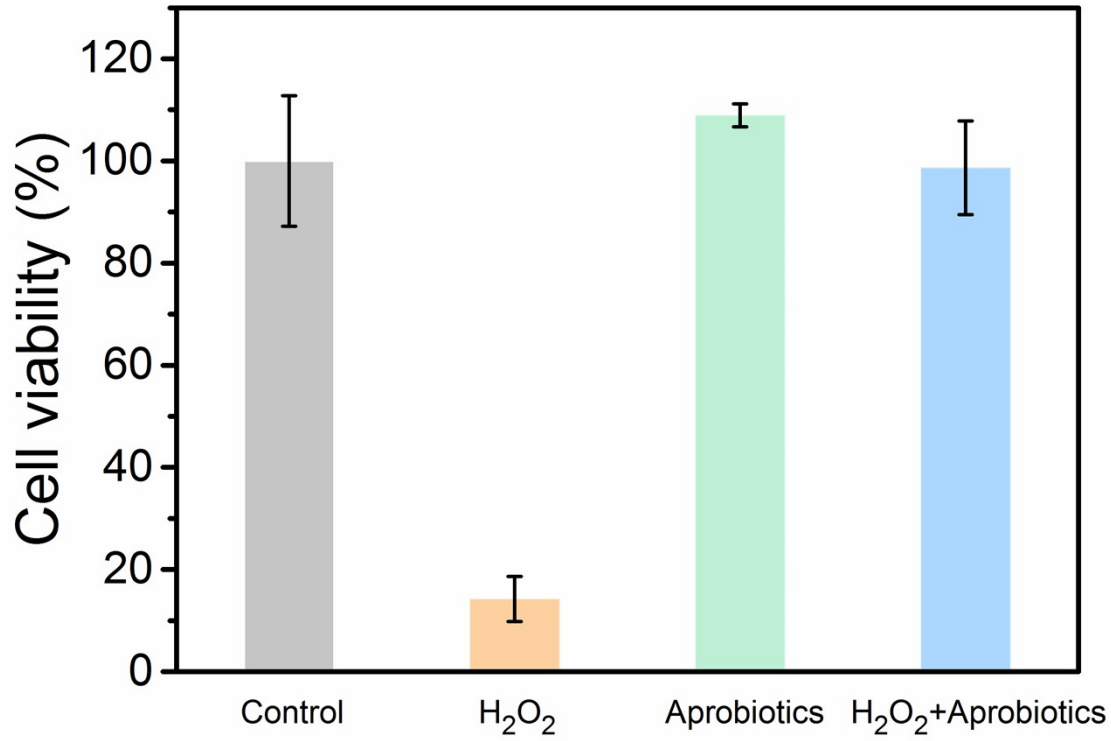

Figure S20 Cell viability of RAW 264.7 after incubated with either H_2O_2 or Aprobiotics.

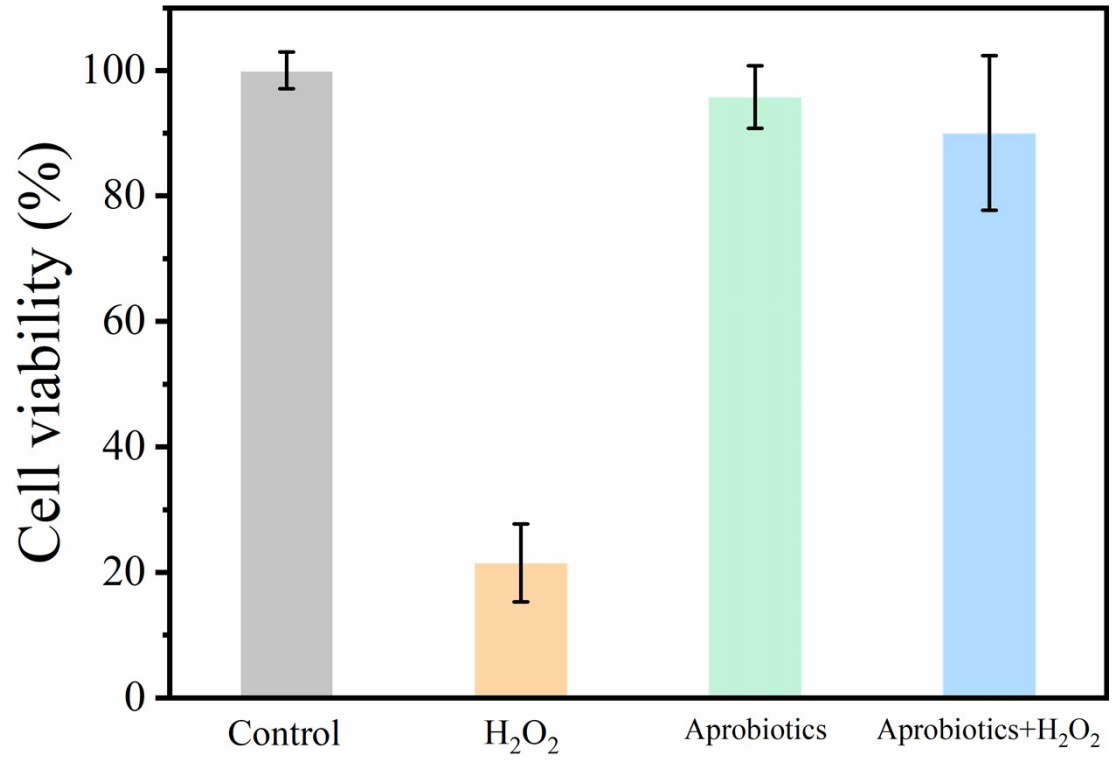

Figure 21 Cell viability of CT26 after incubated with either H_2O_2 or Aprobiotics.

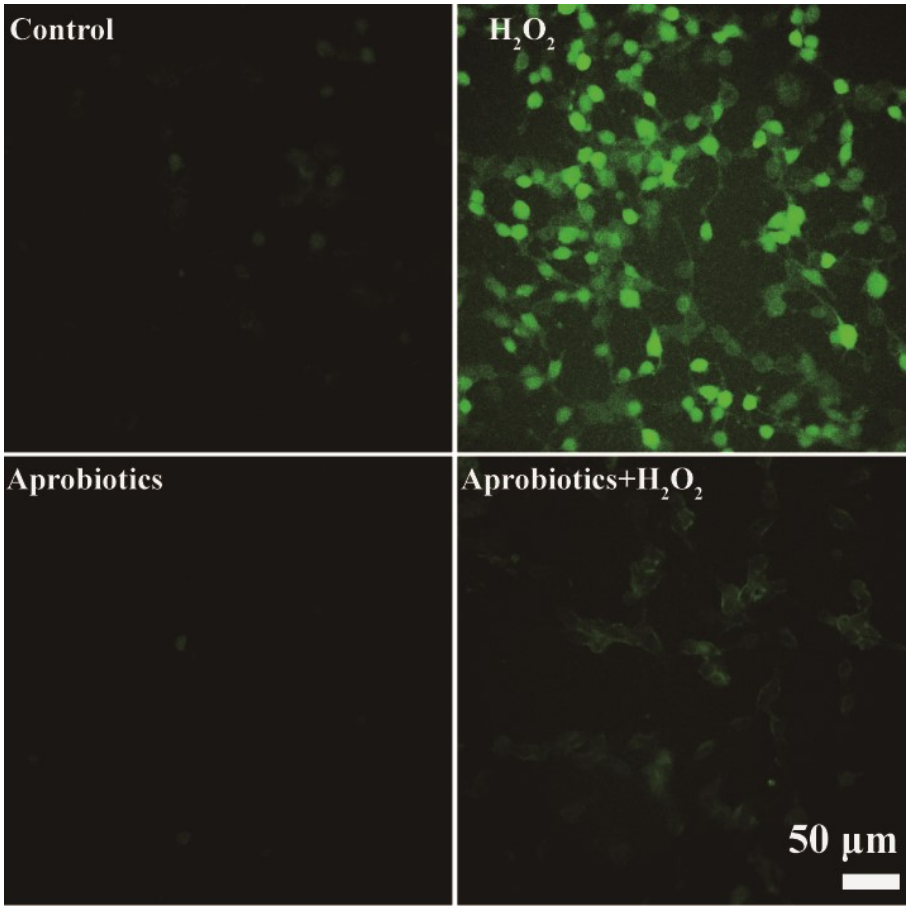

Figure S22 The changes of intracellular ROS according to CLSM images of CT26 cells.

iNOS	forward	GTTCTCAGCCCAACAATACAAGA
	reverse	GTGGACGGGTCGATGTCAC
Arg-1	forward	TGTGTCCAGGCTCCAAATATAG
	reverse	AGCAGGTAGCTGAAGGTCTC
TNF-α	forward	CAGGCGGTGCCTATGTCTC
	reverse	CGATCACCCCGAAGTTCAGTAG
IL-10	forward	ATGCTGCCTGCTCTTACTGACTG
	reverse	CCCAAGTAACCCTTAAAGTCCTG C
GAPDH	forward	CGGAGTCAACGGATTTGGTCGT
	reverse	TCTCAGCCTTGACGGTGCCA

Table S1 Primer sequences used for RT-qPCR.

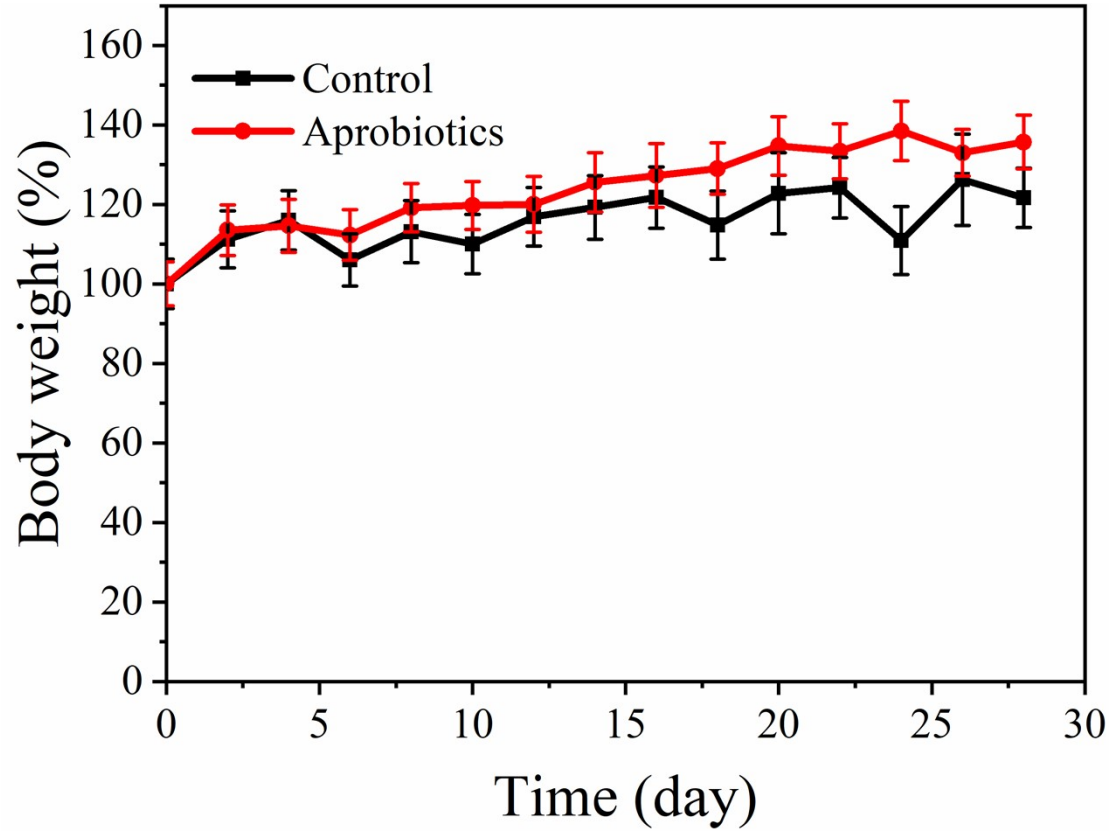

Figure S23 Relative mice body weight after treated with PBS or Aprobiotics.

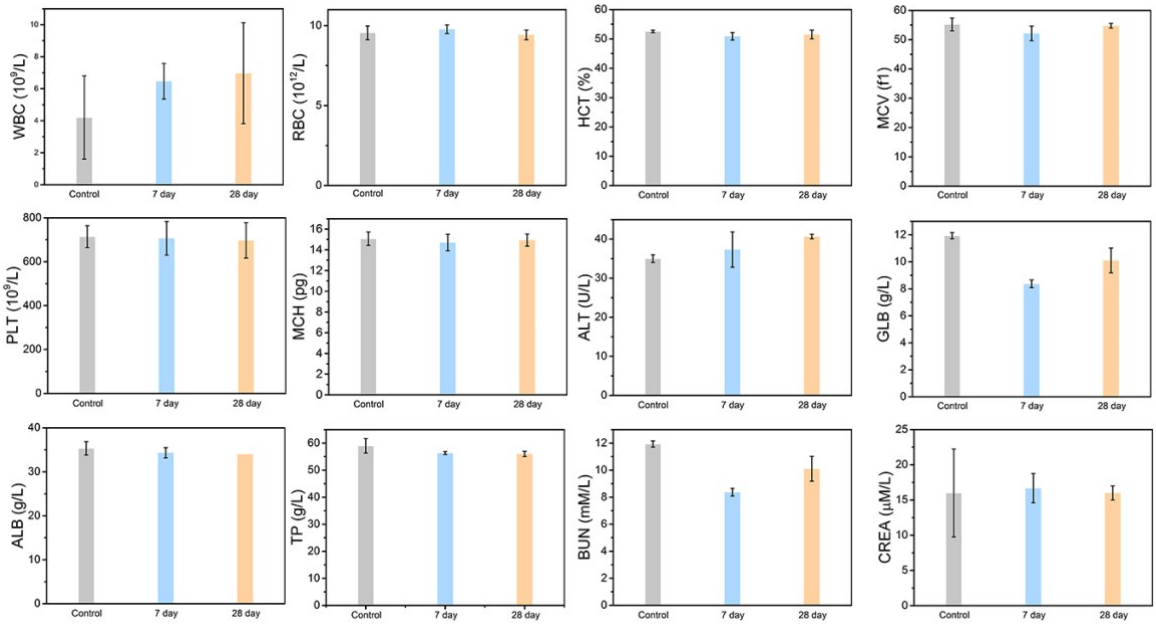

Figure S24 Blood biochemical levels and hematological parameters of the mice after treatment with Aprobiotics for 0, 7 and 28 days.

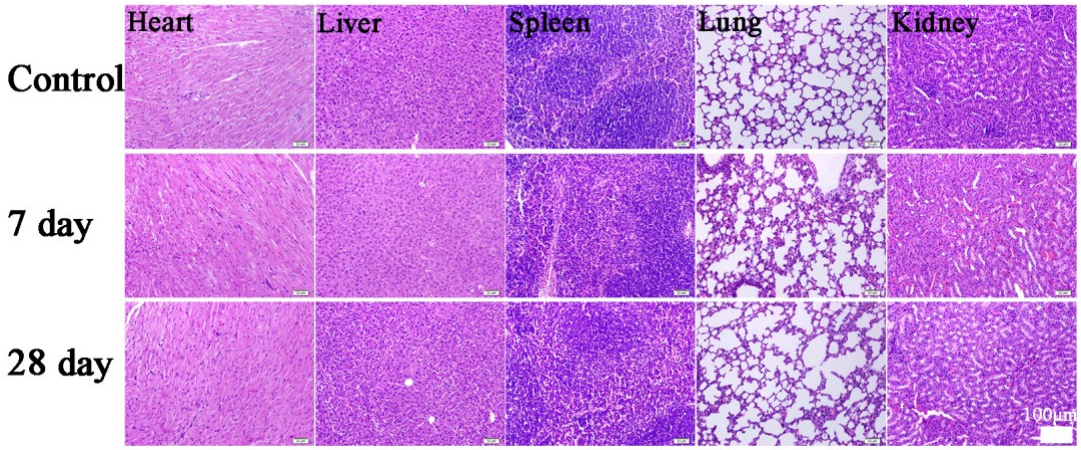

Figure S25 Haematoxylin and eosin staining of the main tissues of Aprobiotics at 0, 7, 28 days post injection.

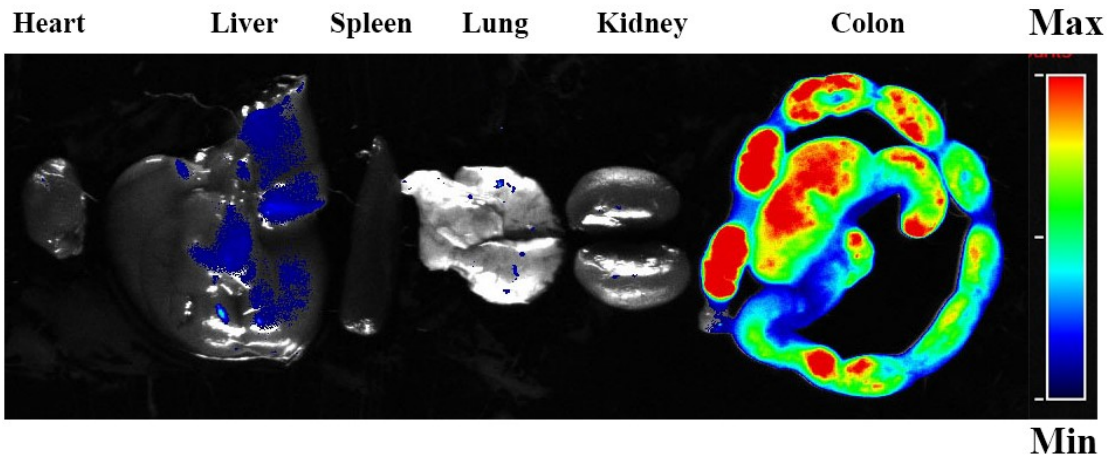

Figure S26 Ex vivo biodistribution fluorescence images of Aprobiotics-Cy3 in major organs and colon from UC model at 6 h.

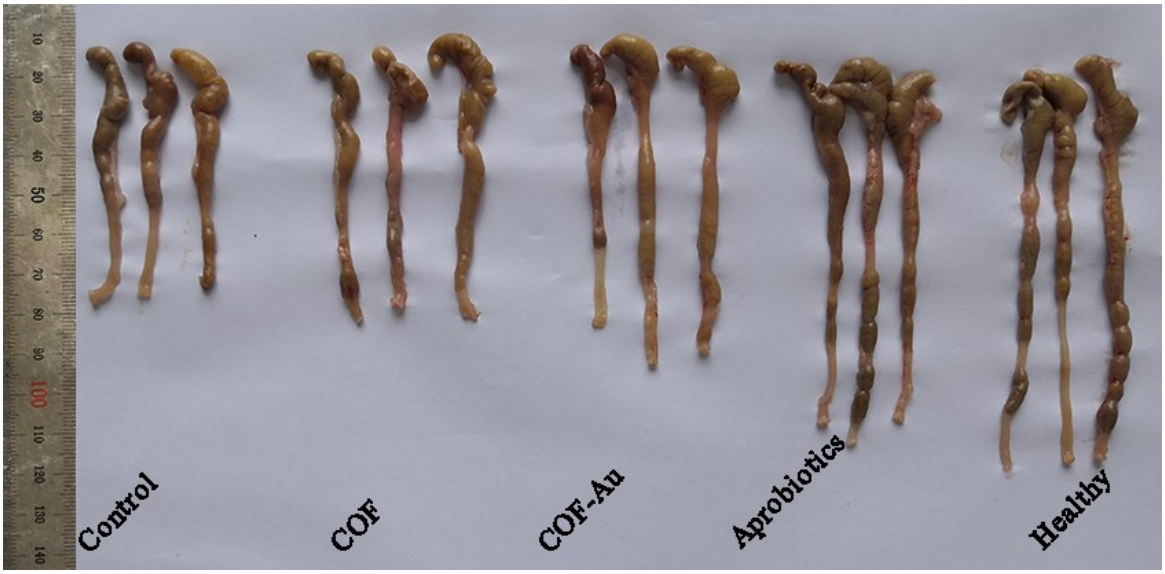

Figure S27 Photos of the colons of every group.