Supporting Information

Acrylate-functionalized Porphyrin-covalent Organic Framework for Bacterial-targeted and Reaction-enhanced Phototherapy/Chemotherapy Synergistic Sterilization and Wound Healing

Meng-Chao Wang,[#123] Jing-Xuan Guo,[#4] Li-Jian Chen,[123] and Xu Zhao,*[123]

[1] State Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi 214122, China.

[2] International Joint Laboratory on Food Safety, Jiangnan University, Wuxi 214122, China.

[3] Institute of Analytical Food Safety, School of Food Science and Technology, Jiangnan University, Wuxi 214122, China.

[4] Key Laboratory of Synthetic and Biological Colloids, Ministry of Education, School of Chemical and Material Engineering Jiangnan University, Wuxi 214122, China.

* E-mail: zhaoxu2017@jiangnan.edu.cn

[#] M.-C. Wang and J.-X. Guo contributed equally to this work.

Supplementary Methods

Chemicals and materials. All chemicals and reagents were at least of analytical grade. 5,10,15,20-tetra-(4-aminophenyl) porphyrin (TAPP) and 2,5-di-(2-methyl methacrylate) p-benzaldehyde (MMA-Da) were purchased from Jilin Chinese Academy of Sciences-Yanshen Technology Co., Ltd. (Jilin, China). *N,N*-Dimethylformamide (DMF), tetrahydrofuran (THF), benzyl alcohol (BnOH), acetonitrile (ACN), Ethanol (EtOH) and acetic acid were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). *N*-butyl alcohol (BuOH), mesitylene, 1,4-Dioxane and 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA) were purchased from Aladdin Chemistry Co., Ltd (Shanghai, China). *o*-dichlorobenzene (*o*-DCB) and 9,10-Anthracenediyl-bis(methylene) dimalonic acid (ABDA) were purchased from Macklin Biochemical Co., Ltd. (Shanghai, China). Ultrapure water was purchased from Wahaha Group Co. (Hangzhou, China). Luria-Bertani (LB) Broth Powder (FMB Grade), LB Agar Powder (FMB Grade) and phosphate buffer saline solution premixed powder were purchased from Shanghai Sangon Biotech (Shanghai, China). Calcein acetoxymethyl ester (Calcein-AM) / Propidium iodide (PI) Double Stain Kit was supplied from Yeason Biotechnology Co. (Shanghai, China). Escherichia coli (*E. coli*, ATCC 25922), Staphylococcus aureus (*S. aureus*, ATCC 25923) were thawed from the frozen bacteria in our laboratory.

Instrumentation and Characterization. Powder X-ray diffraction (PXRD) patterns were recorded on a D2 PHASER X-ray diffractometer (Bruker, German) using Cu Kα radiation (λ = 1.5418 Å) with a scanning speed of 8° min^{-1} and a step size of 0.05° in 2θ. Fourier transform

infrared (FT-IR) spectra were carried out on a Nicolet IS50 spectrometer (Thermo, America) by using KBr pellet. N_2 adsorption experiments were performed on Autosorb-iQ (Quantachrome, USA) using N_2 adsorption at ca. 77 K. The UV-vis absorption spectra were recorded on a UV-3600PLUS (Shimadzu, Japan). Bacteria imaging experiments were carried out on a FLUOVIEW FV3000 confocal laser scanning microscopy (CLSM) (Olympus, Japan). Transmission electron microscopy images (TEM) were obtained at 200 kV with a JEM-2100 TEM (JEOL, Japan). Scanning electron microscopy images (SEM) were obtained with a SU8100 SEM (Hitachi, Japan).

Synthesis of Por-COF*:*

Por-COF was prepared by the following procedure. Briefly, MMA-Da (36.2 mg, 0.12 mmol), TAPP (40.8 mg, 0.06 mmol) and *o*-DCB/BuOH solution (1/9, v/v, 2.0 mL) were added to a Pyrex tube. After ultrasounding for 5 min, 0.2 mL of aqueous acetic acid (6 M) was added to the Pyrex tube with another 5-min sonication. Then, the mixture was degassed in a Pyrex tube through freeze-pump-thaw cycles for three times and then sealed off. Subsequently, the resultant mixture was reacted at 120℃ for 3 days. After cooling down to room temperature, the final product (Por-COF) was collected by centrifugation, washed three times with DMF and THF respectively, and dried at 50℃ under vacuum.

Verification of bonding activity with Sulfhydryl Group*:*

To verify whether the prepared Por-COF can react with adhesins protein under mild conditions, cysteine (Cys) was chosen as an analogue of this protein. For this purpose, Cys

and Por-COF were mixed in phosphate buffer solution (PBS) and stirred at room temperature for 24 h. Then the product was centrifuged, washed three times with water and ethanol, vacuum dried for 12 h, and then characterized by X-ray photoelectron spectroscopy (XPS).

Preparation of Por-COF-Gel*:*

Por-COF-Gel was prepared referring to our previous report with a slight modification.[1] Briefly, Por-COF (20 mg), sodium alginate (SA) (0.5 g) and gelatin (G) (2.0 g) were dispersed in deionized water (50 mL). Then, the mixture was heated at 60℃ and stirred gently until a homogeneous solution was obtained. Finally, Por-COF-Gel was obtained with the concentration of Por-COF was 400 μg mL^{-1}, and stored in dark at 4℃.

Photodynamic Properties of Por-COF*:*

Photodynamic performance of Por-COF was studied with ABDA as the indicator for the generated $^1O_2$. Briefly, 100 μL of ABDA solution (5×10^{-3} mol L^{-1}) and different concentration of Por-COF solution (9.9 mL) were added in a 50 ml centrifuge tube (the final concentration of Por-COF was 0 μg mL^{-1}, 100 μg mL^{-1}, 200 μg mL^{-1}, 400 μg mL^{-1} and 600 μg mL^{-1}, respectively). The above solutions were then irradiated by a white LED light (50, 100 or 150 mW cm^{-2}) for different times (0 min, 3 min, 6 min, 9 min, 12 min, 15 min, 18 min, 21 min, 24 min and 30 min). After that, 1 ml of the irradiated solution was taken out and centrifuged at the abovementioned time point, and the corresponding UV-vis absorption spectra of the suspension were measured by UV-vis-NIR spectrophotometer.

Photothermal Properties of Por-COF*:*

Por-COF was dispersed in PBS at various concentration (100 μg mL^{-1}, 200 μg mL^{-1}, 400 μg mL^{-1} and 600 μg mL^{-1}), then 1 mL of the solution was added into a 48 well cell culture plates followed by irradiation with the white LED light for 30 min (1 mL PBS was used as control). The FLIR-50 thermal camera was used to record the temperature variation during irradiation.

Bacterial Cultures and Sterilization Experiment*:*

Two typical bacteria, Gram-positive *S. aureus* and Gram-negative *E. coli*, were used as the model bacteria to evaluate the bactericidal properties of Por-COF. In briefly, the frozen primitive bacteria were resuscitated and cultured in 5 mL Luria Bertani broth medium under 12-h shaking (200 rpm) at 37℃. Then the bacterial suspension was centrifuged (4000 rpm, 5 min) and resuspended in PBS at a final concentration of 10^8 CFU mL^{-1}.

To evaluate the sterilization ability, the above two model bacteria treated with Por-COF or not were divided into the following groups: Experimental group: The bacterial suspension (*E. coli* or *S. aureus*: 10^8 CFU mL^{-1}, 100 μL) was incubated with Por-COF (final concentrations of 400 μg mL^{-1}) in PBS with shaking (200 rpm) at 37℃ for 30 min. Subsequently, the bacterial suspension was exposed to a white LED light irradiation for different time (0, 10, 20 or 30 min, 100 mW cm^{-2}). Control group: The bacterial suspension cultured without Por-COF and then irradiated with white LED for 30 min (100 mW cm^{-2}). Blank group: The bacterial suspension cultured in PBS without any treatment. After irradiation, the above bacterial suspension was extracted from each group and gradually diluted 10^4 times, respectively. Then, 100 μL of each diluted bacteria solution was evenly spotted onto Luria Bertani broth agar plates and cultured

at 37℃ for 24 h. Finally, the colonies number was counted and the bacterial viability was calculated. Bacterial viability% = (Colonies number of experimental or control group / colonies number of blank group) *100.

The same method was used to evaluate the sterilization ability of Por-COF-Gel.

Staining Analysis of Live/Dead Bacteria*:*

To further confirm the bactericidal effect of Por-COF, the bacteria in abovementioned groups were respectively stained by Calcein-AM / PI Double Stain Kit. After staining for 20 min (2 μmol L^{-1} of Calcein-AM and 4.5 μmol L^{-1} of PI, 100 μL) in the dark, the bacterial suspension was centrifuged and resuspended in 20 μL PBS. Then, 10 μL resuspended bacterial solution was dropped onto the glass slide added with anti-fluorescence quenching sealing agent in advance, fixed for 3–5 min, and observed by CLSM.

Detection of intracellular $^1O_2$ in Bacteria*:*

To further confirm the photodynamic sterilization mechanism of Por-COF, DCFH-DA was used as a detection probe for intracellular $^1O_2$. Typically, 100 μL of bacteria suspension (*E. coli* or *S. aureus*: 10^8 CFU mL^{-1}) was treated with 890 μL Por-COF solution (final concentrations of 400 μg mL^{-1}) and 10 μL DCFH-DA (1 mmol L^{-1}) at 37℃ for 30 min. The blank experiments were performed without Por-COF. After shaking for 30 min, the above bacterial suspension was exposed to a white LED light for different times or not. Then, the bacterial suspension was centrifuged (5000 rpm, 10 min) and resuspended in 20 μL PBS. Finally, 10 μL of re-suspended

bacterial solution was dropped onto the glass slide and then corresponding CLSM images was measured with a 488 nm laser.

Morphological Observation of Bacteria*:*

The bacterial suspensions (100 μL, 10^8 CFU mL^{-1}) were cultured with 900 μL of PBS or Por-COF dispersion (final concentrations of 400 μg mL^{-1}) at 37℃ in the dark for 30 min. Then, the bacterial suspension was irradiated by the white LED light (100 mW cm^{-2}) for 0, 10, 20, 30 min or not, respectively. After that, the suspension was collected by centrifugation (8000 rpm, 5 min) and fixed in 2.5% glutaraldehyde for 20 h. The resulting bacteria were further washed twice with PBS, and dehydrated with a series of ethanol (30%, 50%, 70%, 80%, 95% and 100%, v/v) for 10 min. The bacteria were then frozen at −20℃ and −80℃ for 12 h, respectively, followed by freeze-dried. Finally, the morphology of bacteria was observed by SEM after gold spraying.

Murine Infection Model*:*

Female BALB/c mice (5–6 weeks) were purchased from Changzhou Cavens Experimental Animal Co. Ltd. All animal experiments were carried out strictly in accordance with the protocol approved by the Institutional Animal Care and Use Committee of Jiangnan University (JN.No20220430b0180606[147]). To explore the photothermal/photodynamic/chemical synergistic sterilization and wound healing of Por-COF, *S. aureus* was used to establish an infection mode. First, a round skin wound on the back (d ≈ 7 mm) of each mouse was created. Then, 10 μL of *S. aureus* suspension were dropped on the wound with 24-h infection to offer

the wound infection model. After that, the mice (n = 5) were randomly divided into 6 groups with different treatments: (1) mice with no treatment, (2) mice treated with Gel (50 μL), (3) mice treated with Por-COF-Gel (400 μg mL^{-1}, 50 μL), (4)-(6) The above experimental groups irradiated with 30-min white LED light (100 mW cm^{-2}), respectively. The photographs, body weight and wound area of the mice were recorded and measured every day until the wounds of treatment group of mice were completely healed.

To evaluate the biosafety and therapeutic efficacy of the Por-COF-Gel, the mice were sacrificed after the whole experiment. The wound tissues and main organs (liver, kidney, lung, spleen and heart) were collected and fixed in 4% paraformaldehyde for Masson and hematoxylin and eosin (H&E) staining and immunohistochemistry analysis (including tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6)).

Statistics Analysis*:*

All the quantitative data in each experiment were presented as the mean ± SD (n=3) and P values were calculated by the analysis of variance.

Supplementary References.

1. X. Zhao, X. Wei, L.J. Chen, X.P. Yan, *Biomater. Sci.* 2022, **10**, 2907-2916.

Supplementary Figures

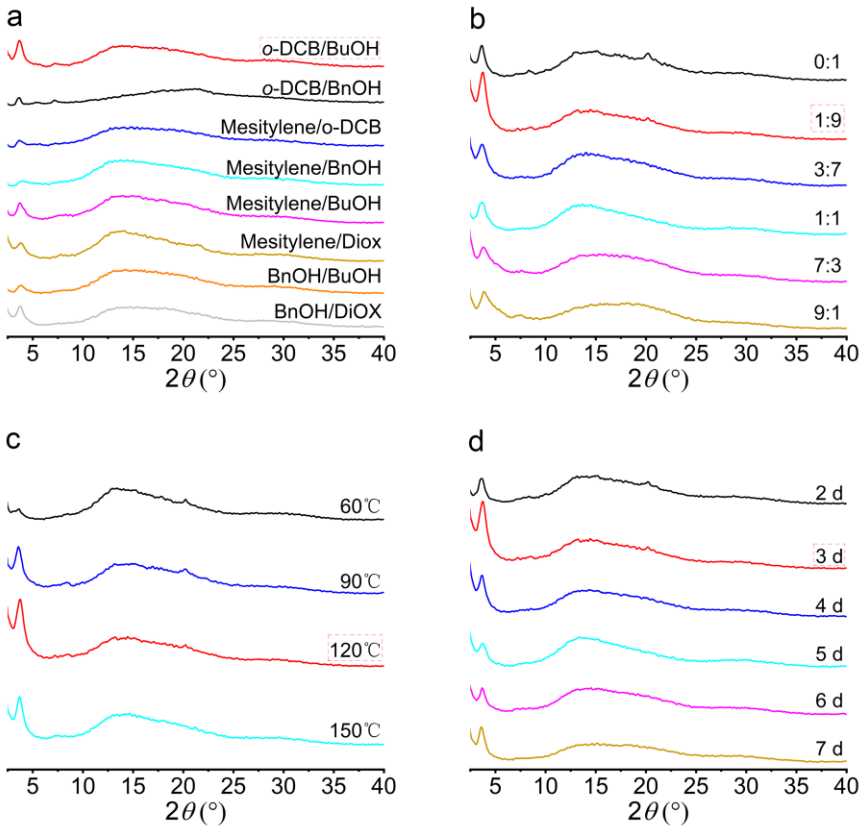

Figure S1. (a) Effect of solvent on the PXRD pattern of Por-COF. (b) Effect of the ratio of *o*-DCB to BuOH (v/v) on the PXRD pattern of Por-COF. (c) Effect of temperature on the PXRD pattern of Por-COF. (d) Effect of time on the PXRD pattern of Por-COF.

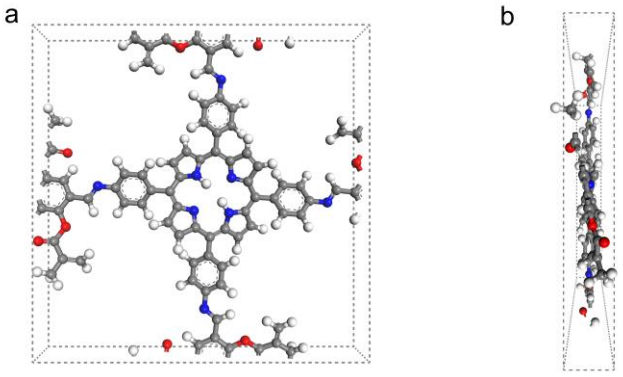

Figure S2. (a) Front view of unit cell of Por-COF in AA stacking model. (b) Side view of unit cell of Por-COF in AA stacking model. (gray C, sky-blue N, red O, white H).

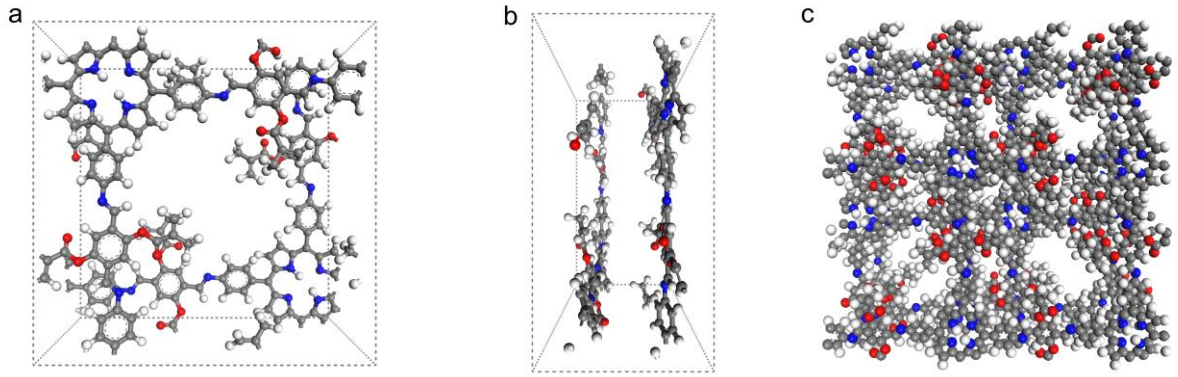

Figure S3. (a) Front view of unit cell of Por-COF in AB stacking model. (b) Side view of unit cell of Por-COF in AB stacking model. (c) Space-filling model of Por-COF in AB stacking model. (gray C, sky-blue N, red O, white H).

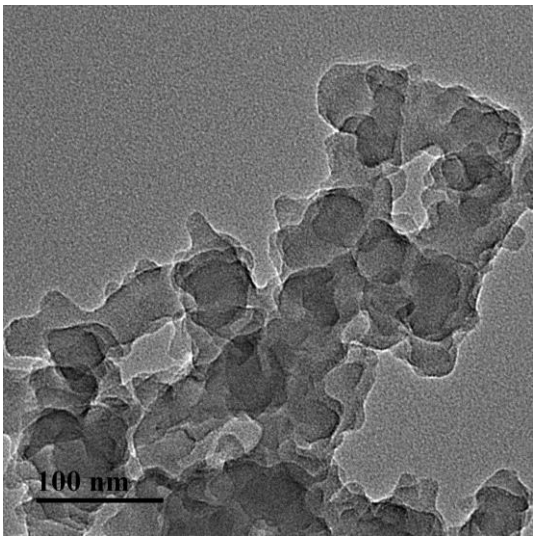

Figure S4. TEM image of Por-COF.

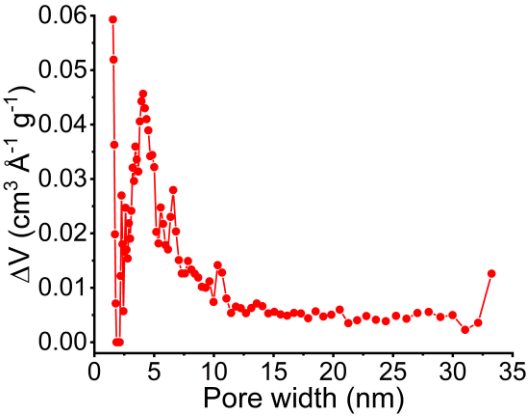

Figure S5. Pore size distribution of Por-COF.

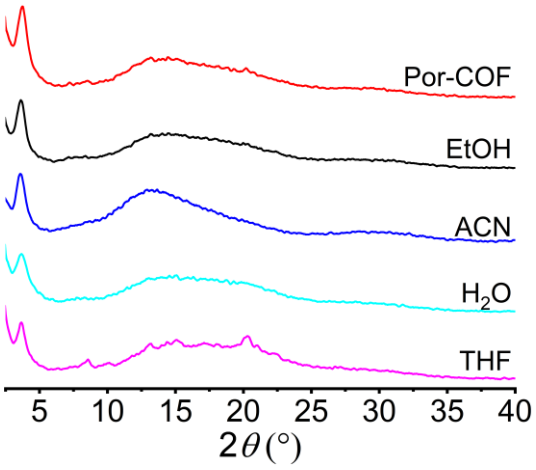

Figure S6. PXRD patterns of Por-COF after treatment with various solvents.

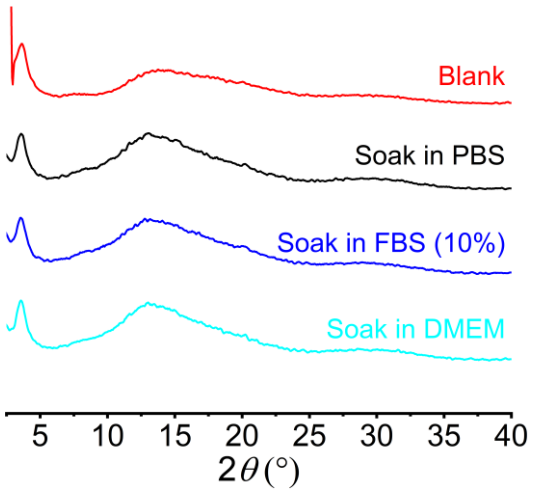

Figure S7. PXRD patterns of Por-COF after soaking in simulated physical environment.

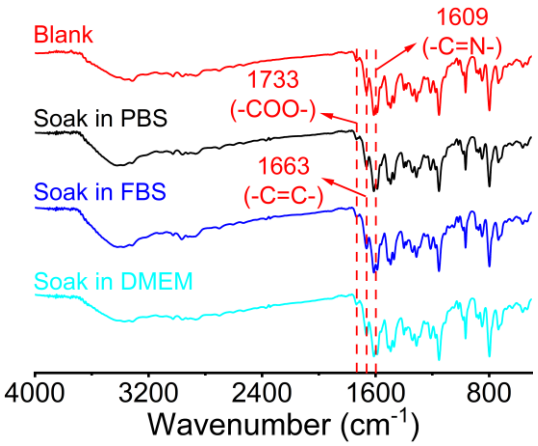

Figure S8. FT-IR spectra of Por-COF after soaking in simulated physical environment.

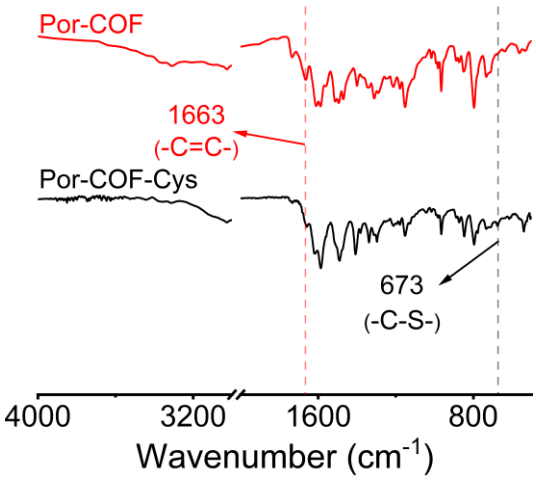

Figure S9. FT-IR spectra of Por-COF and Por-COF-Cys.

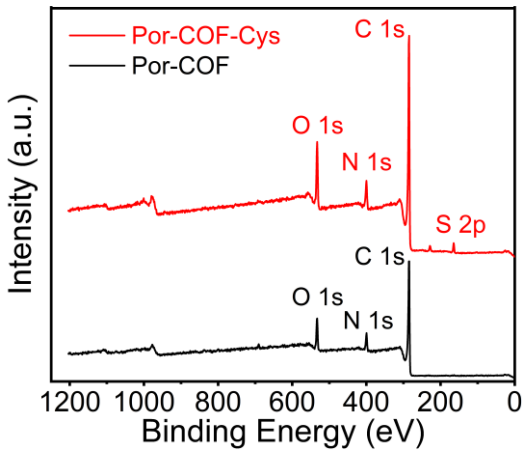

Figure S10. XPS full spectra of Por-COF and Por-COF-Cys.

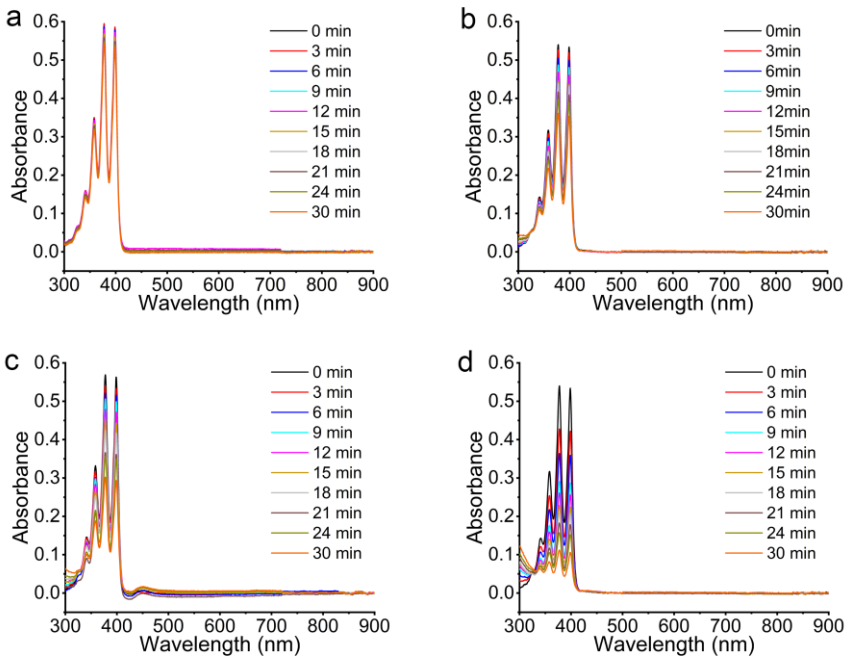

Figure S11. (a, b, c, d) Time-dependent absorption spectra changes of ABDA at 377 nm with different concentrations of Por-COF (a, 0 μg mL^{-1}; b, 100 μg mL^{-1}; c, 200 μg mL^{-1}; d, 600 μg mL^{-1}) under irradiation (100 mW cm^{-2}).

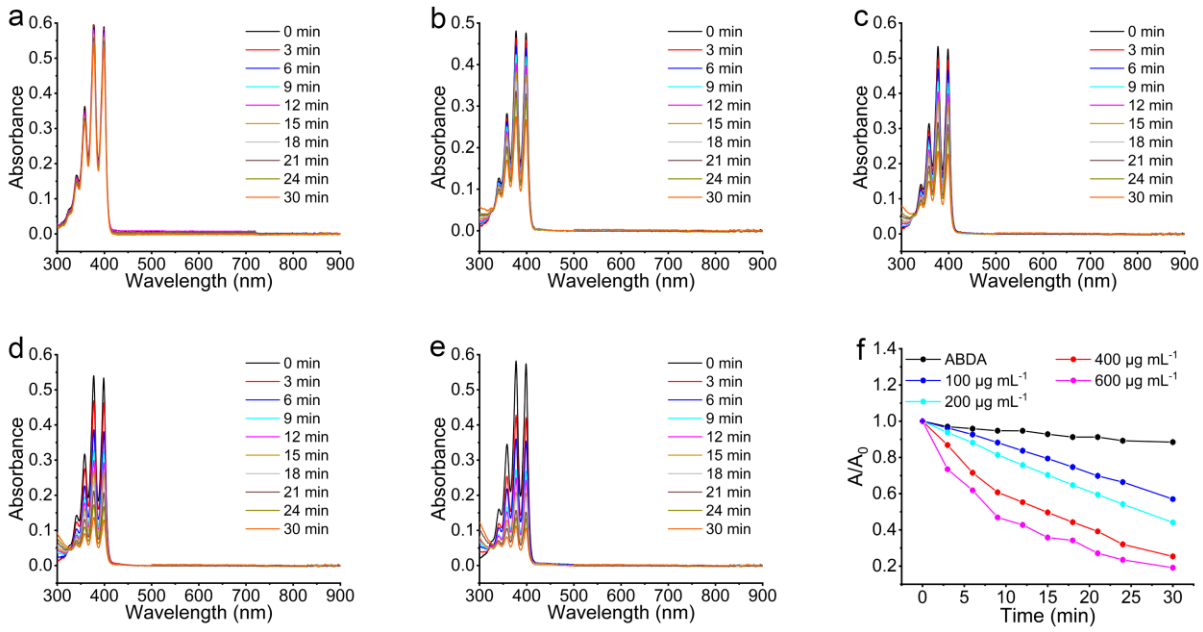

Figure S12. (a, b, c, d, e) Time-dependent absorption spectra changes of ABDA at 377 nm with different concentrations of Por-COF (a, 0 μg mL^{-1}; b, 100 μg mL^{-1}; c, 200 μg mL^{-1}; d, 400 μg mL^{-1}; e, 600 μg mL^{-1}) under irradiation (150 mW cm^{-2}). (f) Time-dependent absorbance of ABDA at 377 nm (A/A_0) with different concentrations of Por-COF under irradiation (150 mW cm^{-2}). A_0 and A are the absorbance of ABDA before irradiation and irradiation at a certain time.

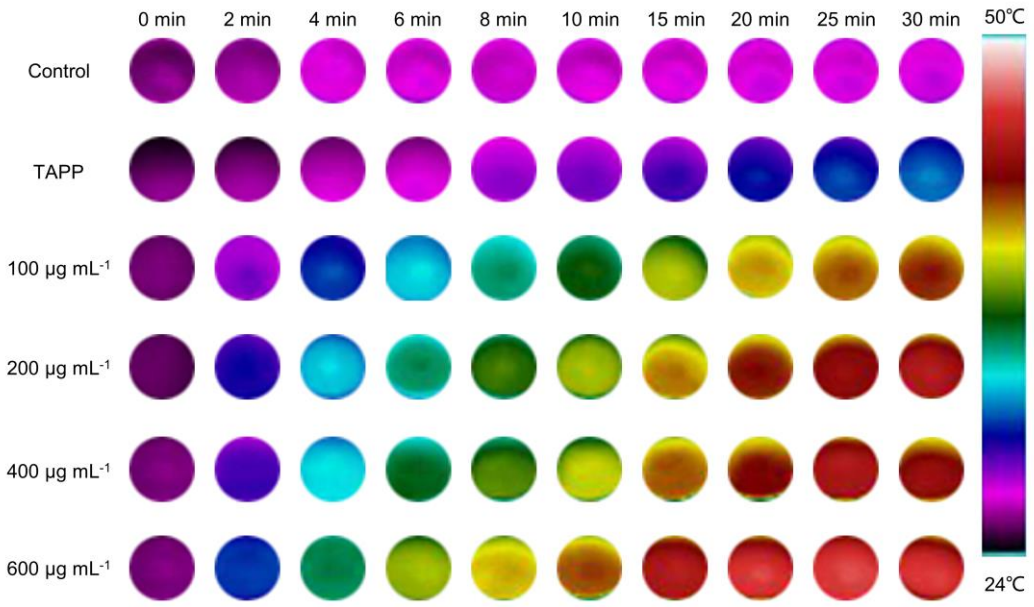

Figure S13. The IR thermal images of different concentrations of Por-COF with irradiation time (100 mW cm^{-2}).

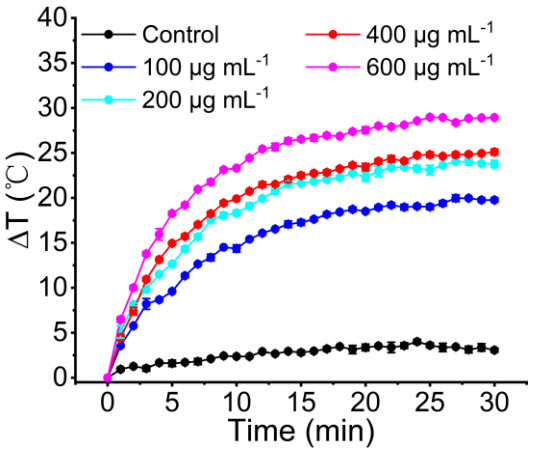

Figure S14. The variation of temperature difference of different concentrations of Por-COF with irradiation time (150 mW cm^{-2})

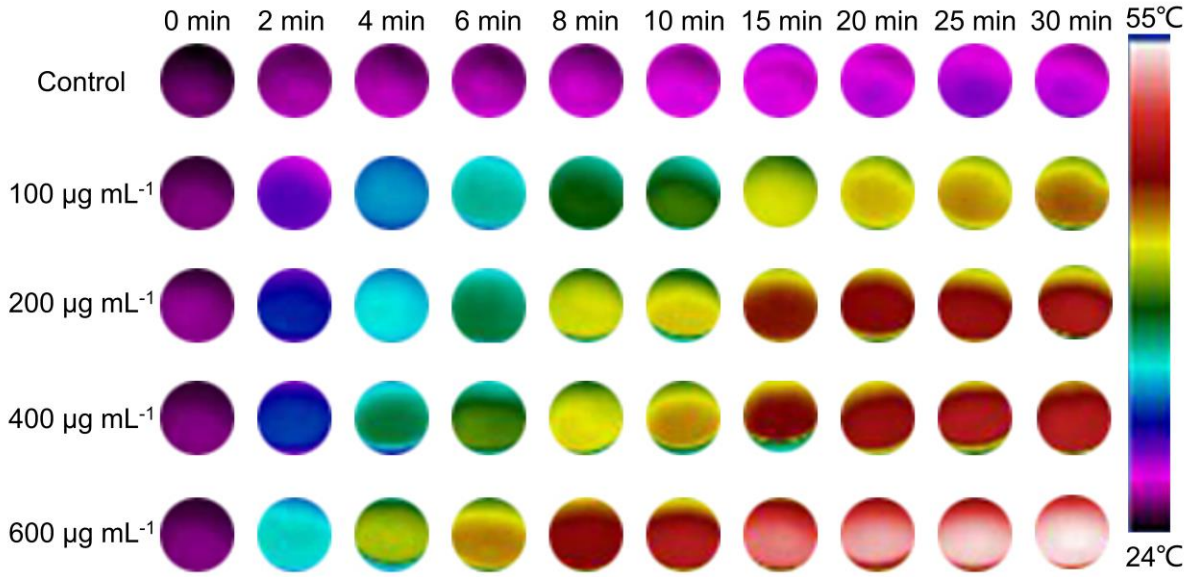

Figure S15. The IR thermal images of different concentrations of Por-COF with irradiation time (150 mW cm^{-2}).

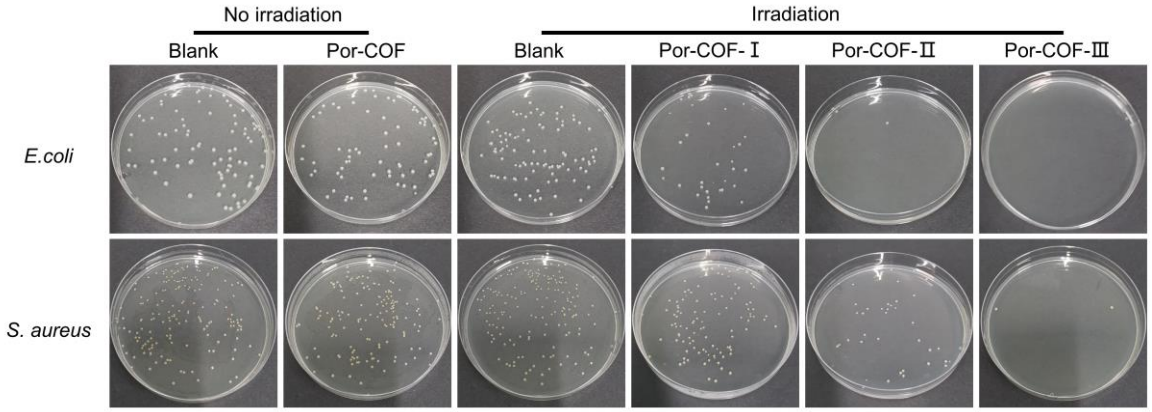

Figure S16. Flat colony photographs of bacterial colonies after different treatments. Por-COF-Ⅰ, Por-COF-Ⅱ and Por-COF-Ⅲ represented irradiation for 10, 20 and 30 min, respectively.

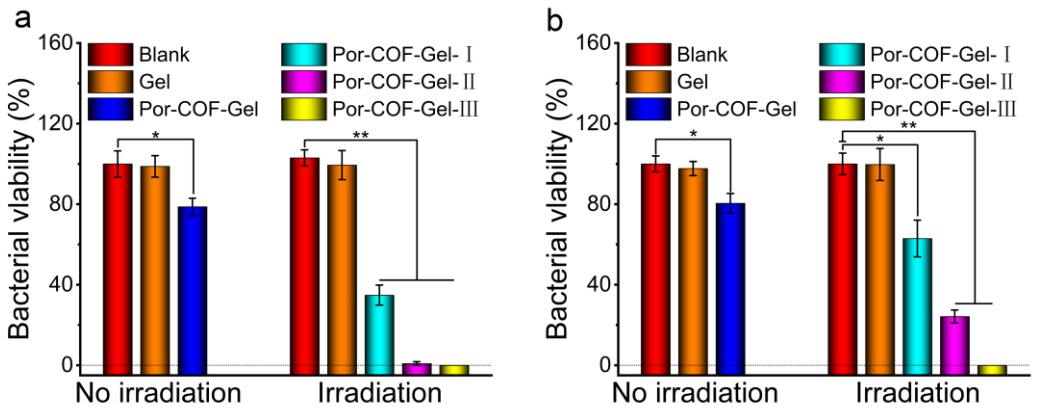

Figure S17. (a) and (b) Bactericidal effect of the Por-COF-Gel (400 μg mL^{-1}) to (a) *E. coli* and (b) *S. aureus* (statistical significance: *, P < 0.05 and **, p < 0.01).

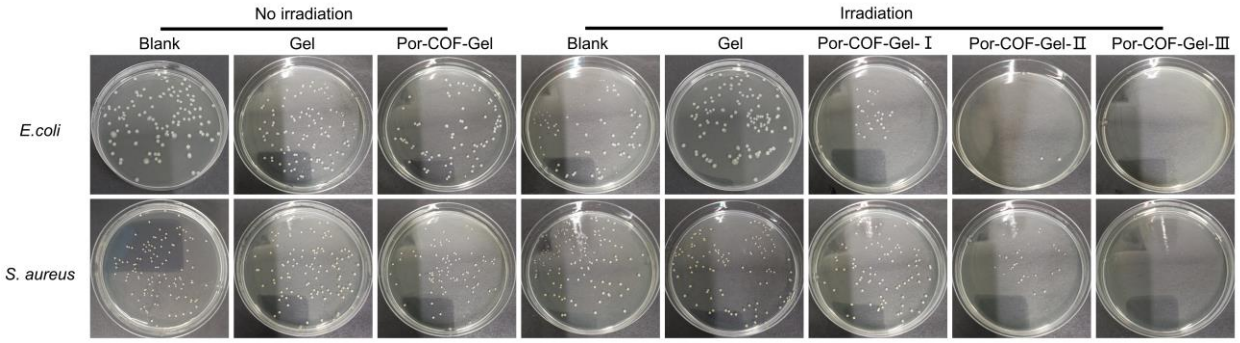

Figure S18. Flat colony photographs of bacterial colonies after different treatments. Por-COF-Gel-Ⅰ, Por-COF-Gel-Ⅱ and Por-COF-Gel-Ⅲ represented irradiation for 10, 20 and 30 min, respectively.

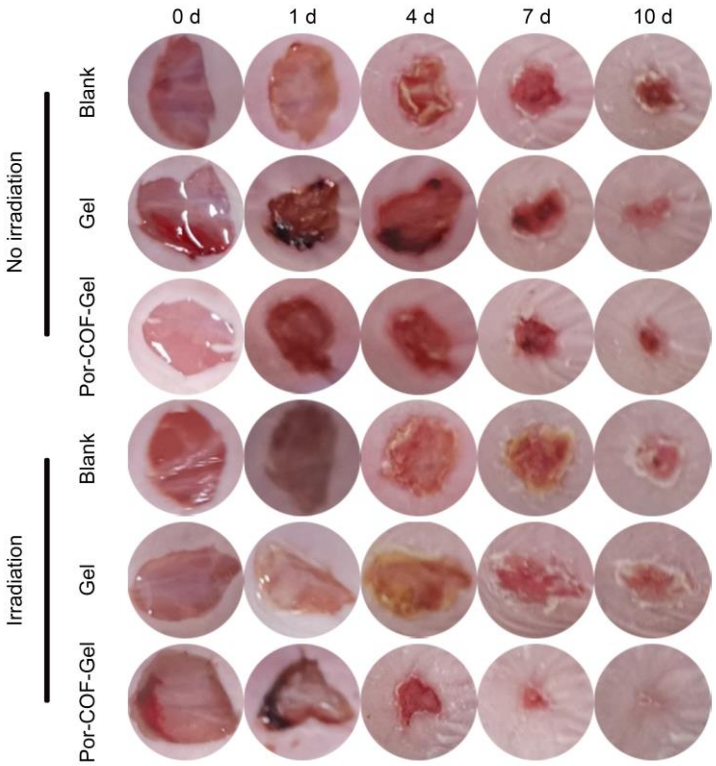

Figure S19. The enlarged image of the wound of the mice of different groups on days 0, 1, 4, 7 and 10 (diameter of circle, 8.5 mm).

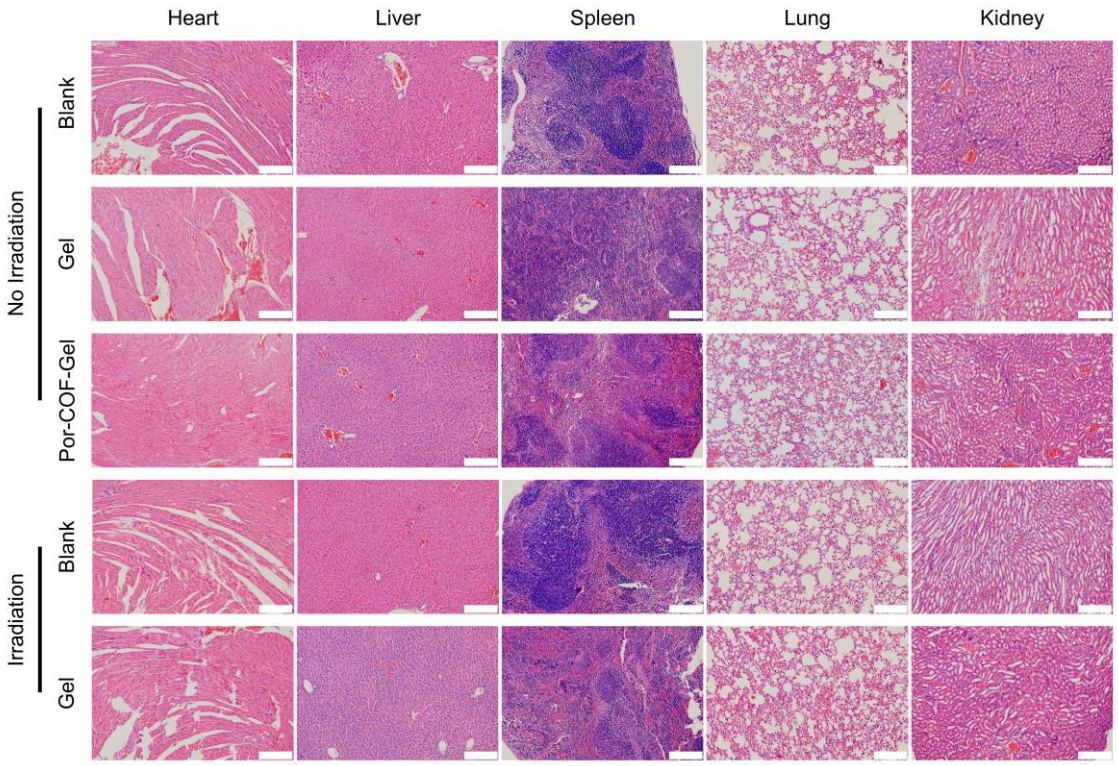

Figure S20. H&E staining of main organs of mice after various treatments for 10 days. (Scale bar, 200 μm).

Supplementary Table

Table S1 Fractional main atomic coordinates for the unit cell of Por-COF after Pawley refinement

Por-COF: Space group symmetry P1/m **a = 25.5478 Å, b = 25.6160 Å, c = 3.6261 Å, α = β = γ = 90°**			
Atom	x	y	z
C1	-0.44281	-0.20072	1.4994
C2	-0.48869	-0.22506	1.62444
C3	-0.49227	-0.27942	1.6305
C4	-0.45056	-0.31069	1.50799
C5	-0.4049	-0.28579	1.38239
C6	-0.40091	-0.2314	1.38057
C7	-0.45476	-0.36913	1.51106
C8	-0.41012	-0.39929	1.59489
C9	-0.36062	-0.378	1.73056
C10	-0.32674	-0.41678	1.72916
C11	-0.35479	-0.46265	1.59269
N12	-0.4052	-0.45068	1.51993
C13	-0.33104	-0.51085	1.50667
C14	-0.27245	-0.51478	1.50387
C15	-0.36147	-0.55551	1.42822
N16	-0.41281	-0.56044	1.50994
C17	-0.42487	-0.61059	1.43278
C18	-0.38188	-0.63694	1.29419
C19	-0.34139	-0.60177	1.29138
C20	-0.47756	-0.69285	1.5146
C21	-0.52337	-0.71771	1.39145
C22	-0.52737	-0.77209	1.3895
C23	-0.48533	-0.80281	1.50582
C24	-0.43933	-0.7785	1.62875
C25	-0.43573	-0.72414	1.63487
C26	-0.47329	-0.63443	1.51666
C27	-0.24143	-0.47329	1.37559
C28	-0.18688	-0.47681	1.37647
C29	-0.16219	-0.52238	1.50243
C30	-0.19268	-0.56398	1.62773
C31	-0.24718	-0.5601	1.63042
N32	-0.4378	-0.14509	1.49207

N33	-0.10642	-0.52731	1.50818
N34	-0.49034	-0.85843	1.49783
C35	-0.51769	-0.60422	1.60592
C36	-0.56686	-0.62552	1.74742
C37	-0.60072	-0.58672	1.75014
C38	-0.57302	-0.54085	1.61009
N39	-0.52278	-0.55283	1.53145
C40	-0.59705	-0.49274	1.5248
C41	-0.6557	-0.48896	1.52613
C42	-0.68677	-0.53095	1.40702
C43	-0.74131	-0.52753	1.40943
C44	-0.76599	-0.48154	1.52727
C45	-0.7355	-0.43938	1.6429
C46	-0.68098	-0.44323	1.6452
N47	-0.82174	-0.47641	1.52519
C48	-0.56681	-0.44813	1.44063
C49	-0.58718	-0.40202	1.30316
C50	-0.54668	-0.36687	1.29897
C51	-0.50338	-0.39305	1.43415
N52	-0.51528	-0.44311	1.51591
C53	-0.07503	-0.4891	1.43086
C54	-0.45092	-0.88959	1.54385
C55	-0.85295	-0.51607	1.49599
C56	-0.47714	-0.11394	1.54224
C57	-0.91	-0.50939	1.48216
C58	-0.94293	-0.55288	1.5222
C59	-0.93154	-0.45984	1.42356
C60	-0.45737	-0.94656	1.53964
C61	-0.41311	-0.97903	1.53325
C62	-0.50755	-0.9685	1.54239
C63	-0.47068	-0.05697	1.53881
C64	-0.42051	-0.03503	1.53713
C65	-0.51495	-0.0245	1.53774
O66	-0.91984	-0.60248	1.57194
C67	-0.95004	-0.64559	1.68732
O68	-0.56495	-0.04797	1.54764
C69	-0.61043	-0.01871	1.47072
O70	-0.3631	-0.95556	1.53869
C71	-0.31776	-0.98483	1.45803
C72	-0.01794	-0.49583	1.44136
C73	0.00361	-0.54538	1.49963

C74	0.01502	-0.45244	1.3966
O75	-0.00805	-0.40289	1.3444
C76	0.02217	-0.35999	1.22577
C77	0.73474	1.03574	0.52049
C78	0.78118	1.0072	0.46197
C79	0.33897	0.95557	0.53622
C80	0.29242	0.98403	0.48032
C81	0.99956	0.69078	0.23282
C82	1.02921	0.73526	0.13906
C83	1.07534	0.30032	0.67496
C84	1.04575	0.25568	0.76595
O85	0.68067	0.96797	0.32556
O86	0.3927	1.02348	0.33912
C87	0.73845	1.08704	0.63554
C88	0.33547	0.90421	0.65045
O89	1.06742	1.63118	0.10523
O90	1.00736	1.35627	0.80527
C91	1.12718	1.2924	0.57528
C92	0.94776	1.69555	0.33273