Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

TCF-based Fluorescent Probe for Monitoring Superoxide Anion Produced in Bacteria Under Chloramphenicol- and Heat-induced Stress

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1. Experimental

General

Apart from the compounds made from chemical reaction, all the auxiliary chemicals used in the study are commercially available. Thin-layer chromatography (TLC) was performed on silica gel plates and visualized by UV to track the reaction. Nuclear Magnetic Resonance (NMR) characterizations were recorded by an Agilent ProPulse 500 spectrometer. Mass Spectrum (MS) analyses were performed using an Agilent QTOF 6545 with Jetstream ESI spray source coupled to an Agilent 1260 Infinity II Quat pump HPLC with 1260 autosampler, column oven compartment and variable wavelength detector (VWD). Fluorescence emission measurements were performed on a BMG Labtech CLARIO star plate reader using Greiner Bio-One microplates (96-well, black-walled). Data were collected via the BMG Labtech Clariostar data analysis software package MARS. UV-Vis absorption measurements were performed on a Varian Cary 500 UV-Vis spectrophotometer. Fluorescence imaging was carried out on a ZEISS LSM-880 Inverted Confocal Laser Scanning Microscope equipped with 561 nm laser source, where samples were prepared on microscopy glass slides. The fluorescence intensities of the images were calculated by Image-J. The pH values were measured on a Hanna Instruments HI 9321 Microprocessor pH meter which was routinely calibrated using Fisher Chemicals standard buffer solutions.

Basic Patterns

Bacterial Strains and Growth Conditions. The strains used include *P. aeruginosa* PAO1, *S. aureus* NCTC 10788, *E. coli* BW 25113, and *E. faecalis* ATCC 29212, which were kindly provided by Professor Toby Jenkins, University of Bath, U.K. Single colonies of *P. aeruginosa*, *S. aureus*, *E. coli* were transferred to Mueller Hinton broth and then incubated with shaking at 37 °C overnight, followed by the subculture in fresh Mueller Hinton broth for the following use. Single colonies of *E. faecalis* were transferred to Brain-Heart Infusion (BHI) broth and then incubated with shaking at 37 °C overnight, followed by the subculture in fresh Brain-Heart Infusion broth for the following use.

Minimum Inhibitory Concentration (MIC). The Minimum Inhibitory Concentrations were determined according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. Briefly, antibiotic was added to a 96 well plate and serially diluted two-fold by Mueller Hinton broth or Brain-Heart Infusion. Overnight cultures of bacteria were sub-cultured in fresh Mueller Hinton broth (or alternatively Brain-Heart Infusion), before adding to all relevant wells in the 96 well plate. Bacteria only and broth only controls was carried out in tandem. After overnight inhibition at 37 ^{o}C, the plates were taken to plate reading to determine the optical densities at 600 nm.

Heating and Probe Incubation Conditions. Heating is performed using a 5×5 EP tube heater with actuate timing and shaking. To determine the mortality caused by heat, planktonic systems were taken to viability test. Probe in pH = 7.40 PBS buffer (10 μM) was co-incubated with planktonic cells after washing and cleaving, under shaking for 1 d at 37 ^{o}C, where they were constantly mixed every 2 hours.

Bacterial Viability Test. Planktonic samples were further diluted to 10^1, 10^2, 10^3, 10^4, and 10^5 times by sterile water. Diluents of each group were all dropped on Mueller Hinton agar plate (or alternatively Brain-Heart Infusion agar plate) for three times, dried, and incubated at 37^{o}C overnight. Then, the numbers of average colony forming units (CFUs) and the standard deviations (between three repetitions) were counted and calculated.

Sample Preparation

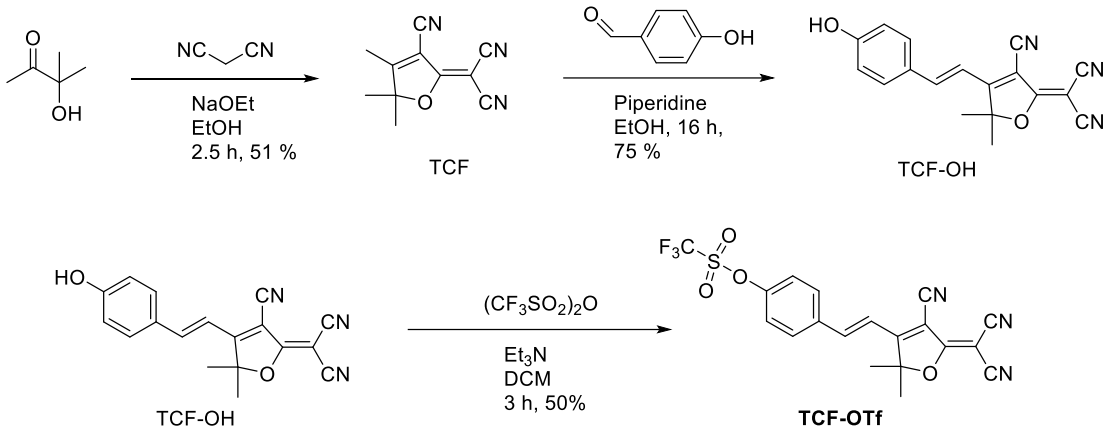
Superoxide Anion ($O_2^{\cdot-}$) and FeTMPyP Testing Solutions. KO_2 (1.0 eq) and 18-crown-6 (2.5 eq) was added to DMSO to afford a final concentration of 1 mM. Then, the mixture was stirred at room temperature for at least 1 h, until all the solids were dissolved to afford a superoxide stock solution. FeTMPyP was stored in freezer and dissolved in DMSO to afford stock solution.

Fluorescence Spectroscopic Samples. Fluorescence Spectrum was performed in 96-well black plates, where the prepared superoxide anion solution was firstly mixed with the DMSO solution of probe. After the reaction is completed, PBS buffer with different pH was further added to the relevant wells to afford a total volume of 200 μL and a probe concentration of 10 μM, then mixed evenly. Meanwhile, blank wells and probe only wells (only probe added by PBS buffer without addition of superoxide anion) were prepared.

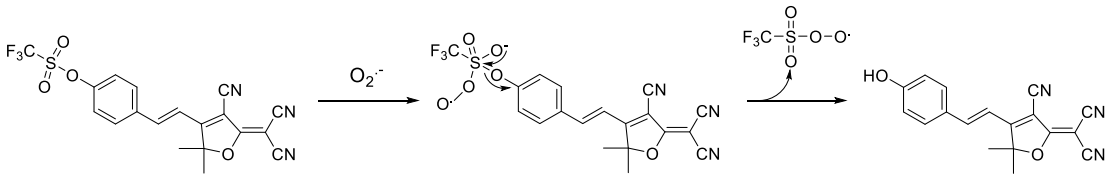
UV-Vis Spectroscopic Samples. UV-Vis spectrum was performed in 96-well clear plates, where the prepared superoxide anion solution was firstly mixed with the DMSO solution of probe. After the reaction is completed, PBS buffer pH = 7.40 was further added to the relevant wells to afford a total volume of 200 μL and a probe concentration of 10 μM, then mixed evenly. Meanwhile, blank wells and probe only wells (only probe added by PBS buffer without addition of superoxide anion) were prepared.

Confocal Laser-Scanning Microscopy (CLSM) Samples. The sub-cultures were allowed to grow in Mueller Hinton broth (or alternatively Brain-Heart Infusion) with or without the presence of Chloramphenicol at 37 ^{o}C for 1 d with presence of probe, under shaking and constant mixing. Alternatively, probe in pH = 7.40 PBS buffer (10 μM) was co-incubated with planktonic cells under shaking and constant mixing for 1 d at 37 ^{o}C. The systems were then taken to centrifugation (14000 rpm, 3 min), and the remaining bacteria were washed with PBS buffer (pH = 7.40), resuspended and placed onto microscope glass slides respectively (covered with cover glass).

Synthesis

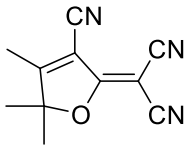


Scheme S1. Synthesis of probe **TCF-OTf.**



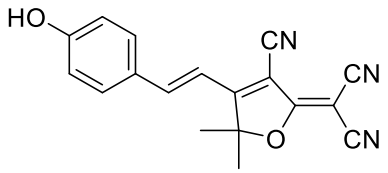
Scheme S2. Proposed sensing mechanism for superoxide anion ($O_2^{\cdot-}$) using **TCF-OTf** based on a non-redox strategy.[2]

2-(3-cyano-4,5,5-trimethylfuran-2(5H)-ylidene)malononitrile (TCF)



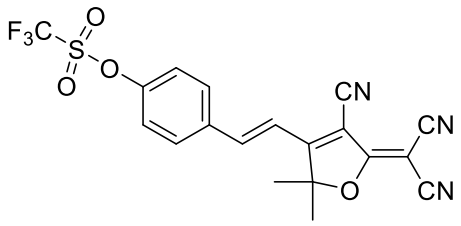
This is made according to the reference.[1] NaOEt (0.391 g, 5.75 mmol) was added to a solution of 3-hydroxy-3-methyl-2-butanone (4 mL, 38 mmol) and malonitrile (4.9 g, 74 mmol) in EtOH (10 mL) and stirred for 1.5 h. The resulting mixture was then stirred at 80 ^{o}C under reflux for 1 h, before cooling down to rt. The mixture was cooled at 0 ^{o}C overnight and the solid precipitate was filtered to afford the title compound as an orange solid (3.83 g, 19.23 mmol, 51 %); ^{1}H NMR (500 MHz, DMSO-D_6) δ 2.36 (s, 3H), 1.59 (s, 6H). ^{13}C NMR (126 MHz, DMSO-D_6) δ 186.2, 177.8, 112.7, 112.0, 110.5, 104.1, 101.8, 55.2, 23.7, 14.7.

(E)-2-(3-cyano-4-(4-hydroxystyryl)-5,5-dimethylfuran-2(5H)-ylidene)malononitrile (TCF-OH)



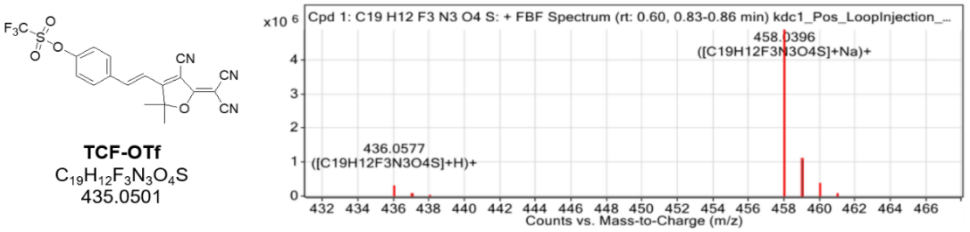
This is made according to the reference.[1] Two drops of Piperidine were added to a mixture of 4-hydroxybenzaldehyde (0.534 g, 4.37 mmol) and TCF (1 g, 5.02 mmol) in EtOH (30 mL). The reaction mixture was stirred at 100 °C under reflux for 16 h, before cooling down to rt. The mixture was cooled at 0 °C overnight and the solid precipitate was filtered to afford the title compound as a red solid (0.995 g, 3.28 mmol, 75 %); ^{1}H NMR (500 MHz, DMSO-D_6) δ 7.89 (d, *J* = 16.2 Hz, 1H), 7.80 (d, *J* = 8.8 Hz, 2H), 7.01 (d, *J* = 16.2 Hz, 1H), 6.89 (d, *J* = 8.5 Hz, 2H), 1.77 (s, 6H). ^{13}C NMR (126 MHz, DMSO-D_6) δ 177.7, 176.3, 162.9, 148.8, 132.8, 126.2, 117.0, 113.4, 112.6, 112.1, 111.7, 99.5, 96.9, 53.6, 25.8.

Trifluoromethanesulfonic 4-[2-(4-cyano-5-dicyanomethylene-2,2-dimethyl-2,5-dihydro-furan-3-yl)-vinyl]-phenyl ester (TCF-OTf)



Trifluoromethanesulfonic anhydride (570 μL, 3.39 mmol) dropwise was added to a solution of TCF-OH (0.344 g, 1.13 mmol) in dry CH_2Cl_2 (DCM, 20 mL) at -78 °C and stirred for 10 min. Triethylamine (565 μL) was then added to the resulting mixture and stirred at rt for 3 h. The solvent was removed under vacuum and the residues were purified through silica column chromatography to afford the title compound as a yellow solid (0.248g, 0.57 mmol, 50 %); M.p. 275 – 278 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.75 (d, *J* = 8.8 Hz, 2H), 7.65 (d, *J* = 16.5 Hz, 1H), 7.42 (d, *J* = 8.8 Hz, 2H), 7.02 (d, *J* = 16.6 Hz, 1H), 1.82 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.6, 151.6, 144.2, 133.9, 130.6, 122.7, 116.7, 111.2, 110.4, 109.8, 101.7, 97.7, 26.3. ^{19}F NMR (470 MHz, $CDCl_3$) δ -72.62 (s). HRMS (ESI-TOF): m/z calculated for $C_{19}H_{12}F_3N_3O_4S$ requires 436.0573 for $[M+H]^+$, found 436.0577.

2. Additional Figures



Compound Table

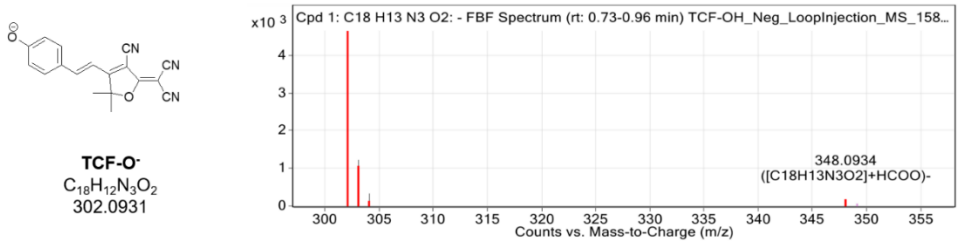
Compound Label	RT (min)	Observed mass (m/z)	Neutral observed mass (Da)	Theoretical mass (Da)	Mass error (ppm)	Isotope match score (%)
Cpd 1: C19 H12 F3 N3 O4 S	0.71	436.0577	435.0505	435.0501	0.91	98.27

Mass errors of between -5.00 and 5.00 ppm with isotope match scores above 60% are considered confirmation of molecular formulae

Peak List

m/z	z	Abund	Formula	Ion
436.0577	1	296681.44	C19H12F3N3O4S	(M+H)+
437.0605	1	67638.96	C19H12F3N3O4S	(M+H)+
438.0582	1	19622.61	C19H12F3N3O4S	(M+H)+
458.0396	1	4872542.5	C19H12F3N3O4S	(M+Na)+
459.0426	1	1075180.13	C19H12F3N3O4S	(M+Na)+
460.0407	1	289411.09	C19H12F3N3O4S	(M+Na)+
461.0416	1	51930.04	C19H12F3N3O4S	(M+Na)+

Figure S1. The high-resolution mass spectrometry (HRMS) of **TCF-OTf**.



Compound Table

Compound Label	RT (min)	Observed mass (m/z)	Neutral observed mass (Da)	Theoretical mass (Da)	Mass error (ppm)	Isotope match score (%)
Cpd 1: C18 H13 N3 O2	0.83	302.0935	303.1007	303.1008	-0.21	84.85

Mass errors of between -5.00 and 5.00 ppm with isotope match scores above 60% are considered confirmation of molecular formulae

Peak List

m/z	z	Abund	Formula	Ion
302.0935	1	4638.8	C18H13N3O2	(M-H)-
303.097	1	1221.36	C18H13N3O2	(M-H)-
304.0989	1	328.54	C18H13N3O2	(M-H)-
348.0934	1	165.17	C18H13N3O2	(M+HCOO)-

Figure S2. The high-resolution mass spectrometry (HRMS) of **TCF-OTf** after reacting with superoxide anion ($O_2^{\cdot-}$).

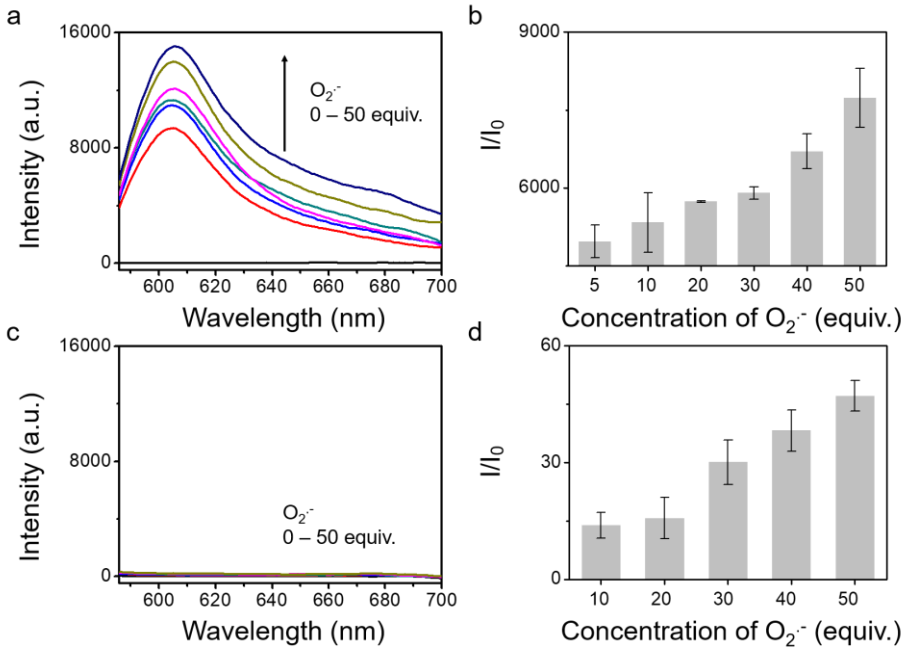


Figure S3. Fluorescence spectra of **TCF-OTf** (10 μM) and corresponding change in fluorescence with addition of $O_2^{\cdot-}$ (0 - 50 equiv.) in PBS buffer solution (10% DMSO, pH = 7.40) (a,b) and in DMSO (c,d). λ_{ex} = 560 nm, λ_{em} = 606 nm.

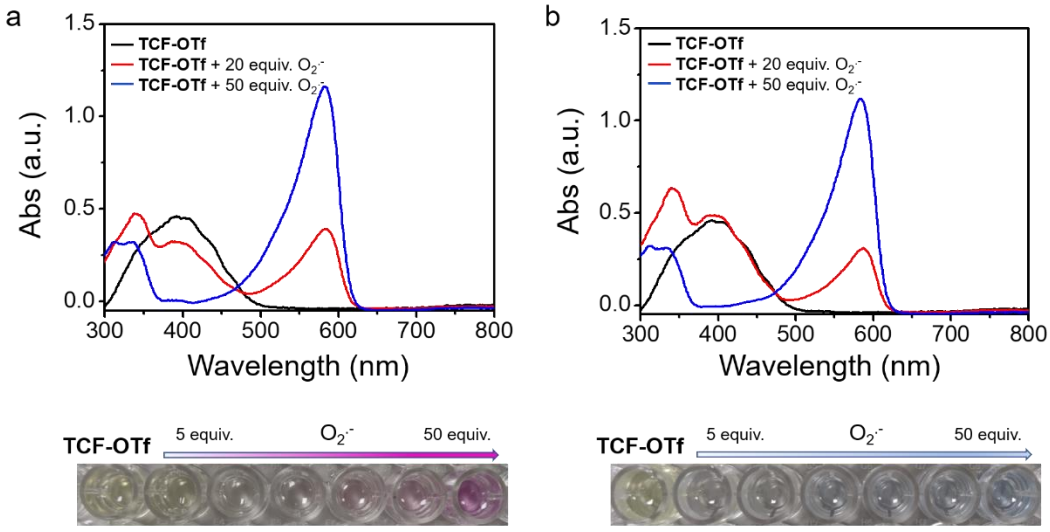


Figure S4. UV-Vis absorption spectrum of **TCF-OTf** (10 μM) with absence or presence of $O_2^{\cdot-}$, and the color change upon gradual addition of $O_2^{\cdot-}$ in PBS buffer solution (10% DMSO, pH = 7.40) (a) and in DMSO (b). To display eye-detectable color change in photographs, the concentrations used are 10-fold higher.

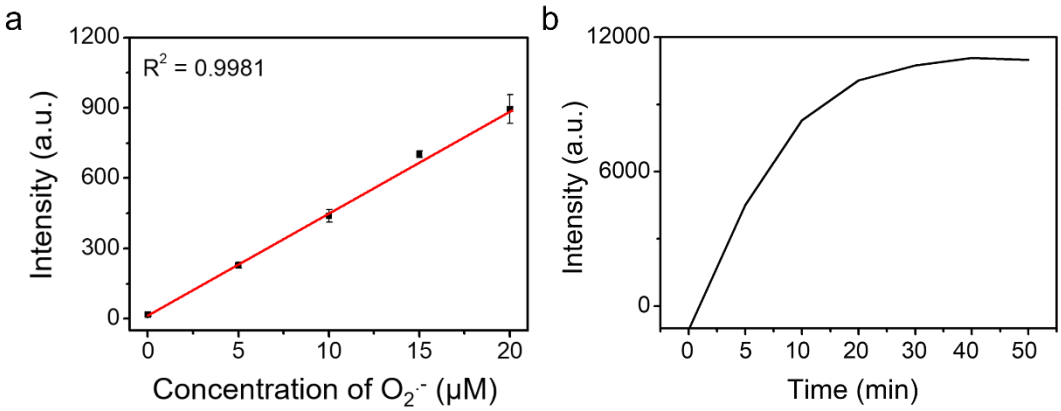


Figure S5. a) Plotting the fluorescence emission changes of **TCF-OTf** (10 μM) as a function of $O_2^{\cdot-}$ concentration for the determination of the limit of detection (3σ/k). b) Time-dependent fluorescence emission changes of **TCF-OTf** (10 μM) with $O_2^{\cdot-}$ (50 equiv.). λ_{ex} = 560 nm, λ_{em} = 606 nm.

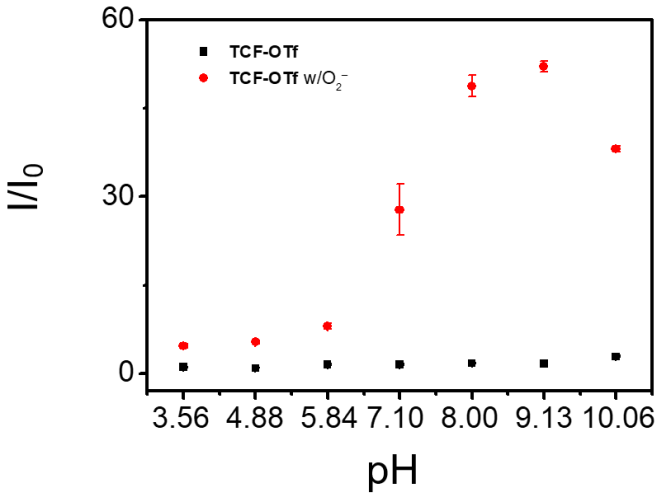


Figure S6. The pH effects on the fluorescence emission of **TCF-OTf** (10 μM) before (black) and after (red) reacting with $O_2^{\cdot-}$ in PBS buffer solutions (10% DMSO). λ_{ex} = 560 nm, λ_{em} = 606 nm.

a

mg/L	500	250	125	62.5	31.3	15.6	7.8	3.9	1.95	0.98
P. aeruginosa	0.172	0.274	0.218	0.325	1.111	1.333	1.475	1.591	1.808	1.599
	0.175	0.204	0.344	0.459	1.179	1.473	1.56	1.793	1.897	1.762
	0.156	0.246	0.331	0.63	1.045	1.437	1.56	1.681	1.902	1.752
S. aureus	0.136	0.257	0.29	0.309	0.736	1.787	1.88	1.813	1.603	1.65
	0.146	0.222	0.245	0.288	0.725	1.67	1.864	1.907	1.643	1.647
	0.128	0.216	0.236	0.2	0.738	1.591	1.804	1.767	1.595	1.455

b

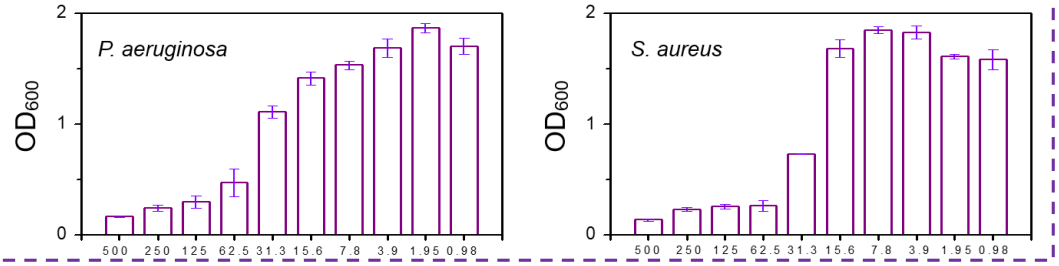


Figure S7. The plate reader detection of OD_{600} absorption intensities of the Minimum Inhibitory Concentration (MIC) well plates and the corresponding bar charts in the MIC trial of Chloramphenicol towards *P. aeruginosa* and *S. aureus*, and the selected sub-MIC wells (yellow) for the subsequent detection by probe **TCF-OTf**.

mg/L	500	250	125	62.5	31.3	15.6	7.8	3.9	1.95	0.98
E. coli	0.431	0.485	0.406	0.417	0.45	0.774	0.457	0.338	1.31	1.911
	0.463	0.508	0.52	0.486	0.492	0.512	0.535	0.416	1.217	1.606
	0.395	0.434	0.469	0.438	0.458	0.794	0.397	0.435	1.172	1.554
E. faecalis	0.495	0.783	0.896	0.939	1.089	2.058	2.294	2.414	1.329	1.263
	0.511	0.843	1.019	1.08	1.158	2.127	2.38	2.457	1.549	1.607
	0.471	0.827	1.044	1.036	1.157	1.899	2.242	2.18	1.371	1.463

mg/L	500	250	125	62.5	31.3	15.6	7.8	3.9	1.95	0.98
E. coli	0.512	0.568	0.493	0.501	0.514	0.504	0.496	0.406	1.325	1.716
	0.384	0.412	0.405	0.347	0.382	0.565	0.42	0.328	1.3	1.724
	0.377	0.501	0.434	0.411	0.462	0.77	0.625	0.558	1.389	2.031
E. faecalis	0.525	0.786	1.003	1.03	1.114	2.13	2.368	2.46	1.294	1.491
	0.689	0.746	0.847	0.905	1.136	2.359	2.525	2.258	1.443	1.446
	0.453	0.707	0.763	0.843	0.919	2.124	2.369	2.481	1.325	1.201

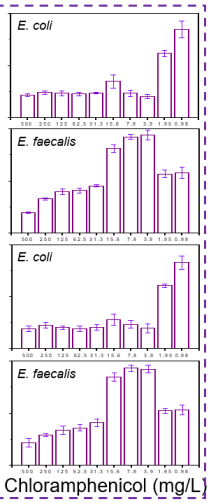


Figure S8. The plate reader detection of OD_{600} absorption intensities of the Minimum Inhibitory Concentration (MIC) well plates and the corresponding bar charts in the MIC trial of Chloramphenicol towards *E. coli* and *E. faecalis*, and the selected sub-MIC wells (yellow) for the subsequent detection by probe **TCF-OTf**.

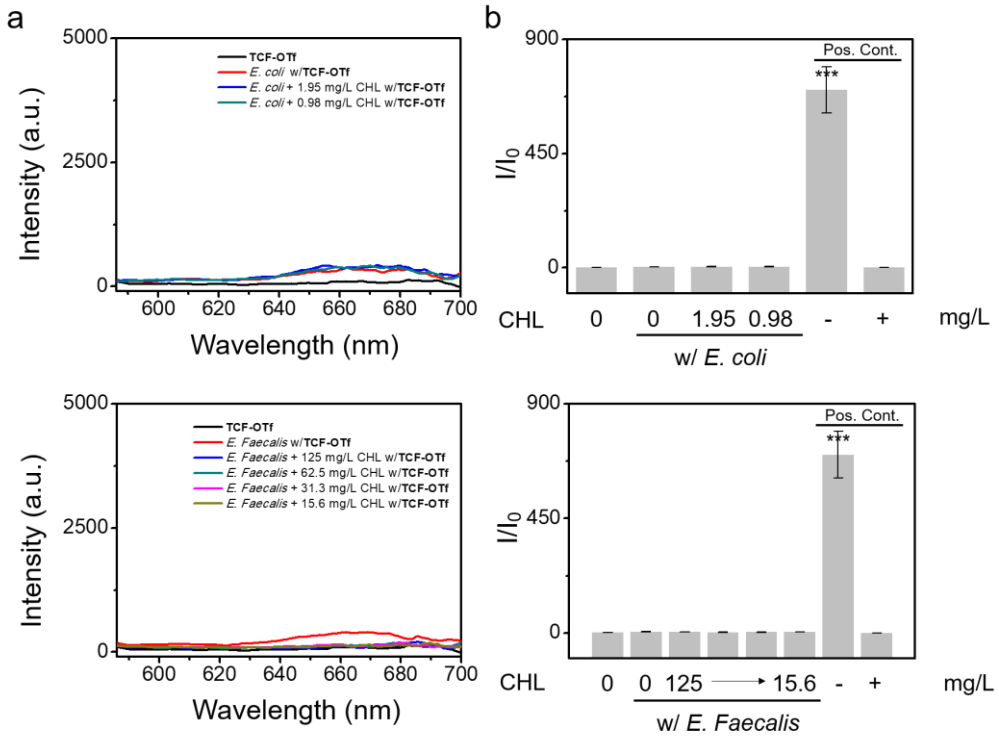


Figure S9. TCF-OTf based fluorescence detection (a) and corresponding change in fluorescence (b) with *E. coli* and *E. faecalis* without antibiotic treatment (control), and *E. coli*, *E. faecalis* picked from the MIC plate wells under treatment at sub-MIC concentrations with chloramphenicol (CHL). Given that no clear signal was detected, a positive control was used (-: **TCF-OTf** + $O_2^{\cdot-}$; +: **TCF-OTf** + $O_2^{\cdot-}$ + FeTMPyP) to prove that the probe was still working. ***P<0.001. λ_{ex} = 560 nm, λ_{em} = 606 nm.

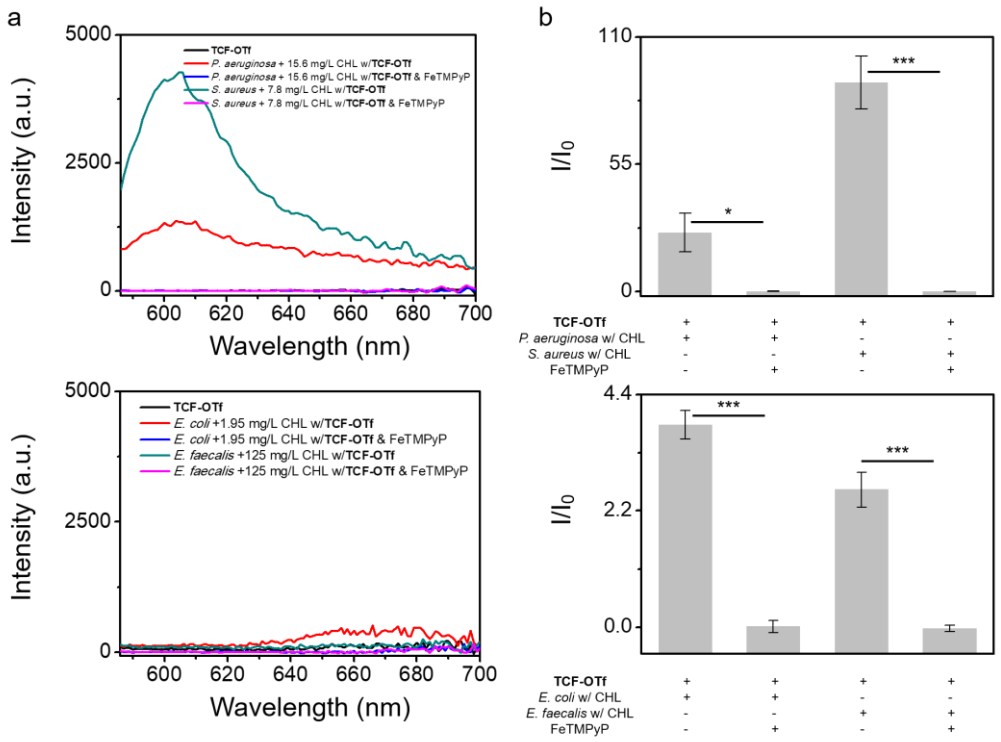


Figure S10. TCF-OTf based fluorescence emission spectrum detection and corresponding change in fluorescence with presence of *P. aeruginosa*, *S. aureus*, *E. coli*, and *E. faecalis* with treatment of sub-MIC concentrations of Chloramphenicol (CHL), and with or without the further treatment of FeTMPyP. *P<0.02, ***P<0.001. λ_{ex} = 560 nm, λ_{em} = 606 nm.

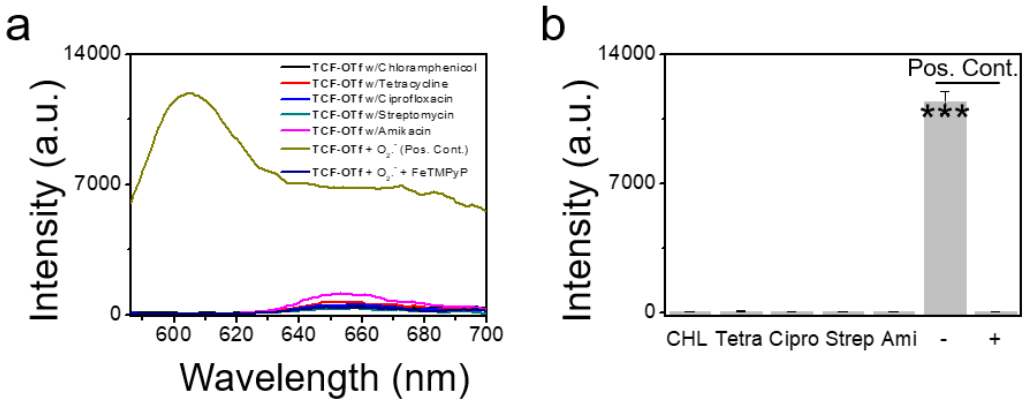


Figure S11. The solutional response of **TCF-OTf** towards antibiotics and corresponding change in fluorescence. Positive control was used (-: **TCF-OTf** + $O_2^{\bullet-}$; +: **TCF-OTf** + $O_2^{\bullet-}$ + FeTMPyP) for comparison. ***P<0.001. λ_{ex} = 560 nm, λ_{em} = 606 nm.

mg/L	500	250	125	62.5	31.3	15.6	7.8	3.9	1.95	0.98
Tetracycline towards *P. aeruginosa*	0.283	0.216	0.158	0.343	0.988	1.394	1.883	1.638	1.68	1.657
	0.987	0.219	0.169	0.293	0.927	1.286	1.814	1.694	1.76	1.77
	0.237	0.235	0.176	0.181	0.967	1.384	1.742	1.952	1.859	1.977
Tetracycline towards *S. aureus*	0.338	0.234	0.277	0.184	0.159	0.136	0.136	0.139	1.416	1.714
	0.268	0.255	0.195	0.185	0.173	0.177	0.182	0.486	1.489	2.348
	0.301	0.247	0.228	0.195	0.193	0.131	0.195	0.629	1.492	1.814
mg/L	**5**	**2.5**	**1.25**	**0.625**	**0.313**	**0.156**	**0.078**	**0.039**	**0.0195**	**0.0098**
Ciprofloxacin towards *P. aeruginosa*	0.127	0.132	0.123	0.101	0.205	0.276	1.063	1.447	1.745	1.985
	0.101	0.111	0.128	0.097	0.092	0.148	1.128	1.497	1.852	1.77
	0.088	0.105	0.145	0.101	0.093	0.11	1.196	1.802	2.058	2.253
Ciprofloxacin towards *S. aureus*	0.531	0.877	0.873	0.622	0.285	0.317	0.543	1.781	2.152	1.973
	0.371	0.9	1.143	0.807	0.523	0.375	0.663	2.481	3.061	3.407
	0.731	1.392	1.268	0.802	0.463	0.241	1.936	3.59	3.919	4.003

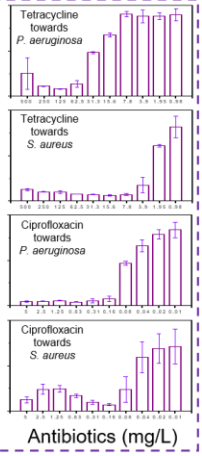


Figure S12. The plate reader detection of OD_{600} absorption intensities of the MIC well plates and the corresponding bar charts in the MIC trial of Tetracycline and Ciprofloxacin towards *P. aeruginosa* and *S. aureus*, and the selected sub-MIC wells (yellow) for the subsequent detection (screening antibiotics) by probe **TCF-OTf**.

mg/L	500	250	125	62.5	31.3	15.6	7.8	3.9	1.95	0.98
Streptomycin towards *P. aeruginosa*	0.29	0.265	0.227	0.228	0.242	0.207	0.892	1.686	1.411	1.383
	0.287	0.247	0.187	0.192	0.199	0.193	0.765	1.827	1.346	1.357
	0.261	0.204	0.194	0.176	0.194	0.196	0.828	1.731	1.553	1.455
Streptomycin towards *S. aureus*	0.252	0.222	0.21	0.179	0.176	0.205	0.756	1.464	2.128	1.503
	0.074	0.136	0.109	0.083	0.06	0.054	0.736	1.347	1.996	1.539
	0.225	0.197	0.171	0.174	0.137	0.144	0.524	1.053	1.661	1.171
mg/L	**250**	**125**	**62.5**	**31.3**	**15.6**	**7.8**	**3.9**	**1.95**	**0.98**	**0.49**
Amikacin towards *P. aeruginosa*	0.234	0.21	0.207	0.213	0.195	0.209	0.188	0.191	0.236	0.381
	0.199	0.215	0.252	0.182	0.19	0.187	0.199	0.183	0.149	0.385
	0.209	0.192	0.182	0.197	0.18	0.178	0.186	0.182	0.145	0.501
Amikacin towards *S. aureus*	0.225	0.231	0.235	0.237	0.245	0.254	0.264	0.196	0.236	1.032
	0.185	0.21	0.218	0.183	0.182	0.269	0.201	0.204	0.438	0.715
	0.173	0.179	0.201	0.216	0.236	0.216	0.207	0.239	0.357	0.969
mg/L	**0.24**	**0.12**	**0.06**	**0.03**	**0.015**	**0.0076**	**0.0038**	**0.0019**	**0.001**	**0.0005**
Amikacin towards *P. aeruginosa*	2.566	1.871	2.136	2.109	1.342	1.604	1.609	1.541	1.627	1.672
	1.968	2.741	1.949	2.137	1.827	1.75	1.709	1.712	1.616	1.581
	2.638	2.834	1.889	1.961	1.911	1.892	1.683	1.664	1.622	1.801
Amikacin towards *S. aureus*	1.44	1.776	1.576	1.543	1.351	1.434	1.553	1.637	1.597	1.387
	1.959	1.641	1.545	1.612	1.351	1.441	1.499	1.515	1.504	1.383
	2.008	1.534	1.395	1.214	1.222	1.095	1.235	1.11	1.102	1.088

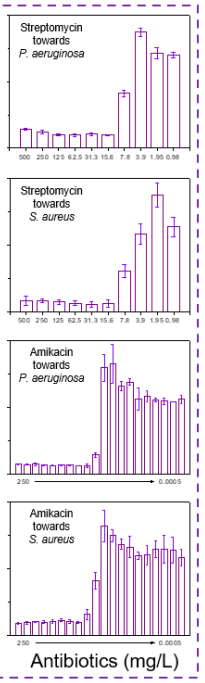


Figure S13. The plate reader detection of OD_{600} absorption intensities of the MIC well plates and the corresponding bar charts in the MIC trial of Streptomycin and Amikacin towards *P. aeruginosa* and *S. aureus*, and the selected sub-MIC wells (yellow) for the subsequent detection (screening antibiotics) by probe **TCF-OTf**.

mg/L	500	250	125	62.5	31.3	15.6	7.8	3.9	1.95	0.98
Tetracycline towards *E. coli*	0.425	0.373	0.388	0.58	0.58	0.528	0.494	0.509	0.694	2.038
	0.335	0.426	0.282	0.616	0.511	0.478	0.445	0.48	0.844	2.034
	0.389	0.411	0.345	0.628	0.493	0.45	0.43	0.453	0.744	2.102
Tetracycline towards *E. faecalis*	0.344	0.301	0.34	1.268	1.407	1.203	1.745	2.414	2.735	2.712
	0.327	0.285	0.396	1.597	1.465	1.452	1.655	2.344	2.678	2.691
	0.312	0.29	0.325	1.192	1.174	1.527	2.228	2.651	2.835	2.885
mg/L	**500**	**250**	**125**	**62.5**	**31.3**	**15.6**	**7.8**	**3.9**	**1.95**	**0.98**
Ciprofloxacin towards *E. coli*	0.19	0.702	0.475	0.473	0.424	0.427	0.407	0.371	0.237	0.149
	0.221	0.732	0.486	0.509	0.504	0.493	0.481	0.429	0.297	0.145
	0.221	0.735	0.482	0.518	0.517	0.537	0.512	0.456	0.349	0.164
Ciprofloxacin towards *E. faecalis*	0.327	0.233	0.194	0.132	0.105	0.204	2.236	2.372	2.71	2.786
	0.427	0.329	0.334	0.306	0.161	0.247	1.986	2.377	2.622	2.825
	0.6	0.431	0.4	0.309	0.183	0.11	2.369	2.792	2.896	3.049
mg/L	**0.245**	**0.123**	**0.061**	**0.031**	**0.015**	**0.008**	**0.004**	**0.002**	**0.001**	**0.0005**
Ciprofloxacin towards *E. coli*	0.165	0.193	0.189	0.174	0.178	0.175	0.176	1.183	1.131	1.064
	0.209	0.207	0.204	0.185	0.181	0.198	0.186	1.27	1.194	1.105
	0.161	0.172	0.185	0.155	0.144	0.171	0.202	1.106	1.064	1.108

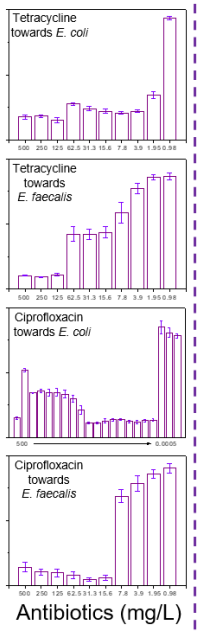


Figure S14. The plate reader detection of OD_{600} absorption intensities of the MIC well plates and the corresponding bar charts in the MIC trial of Tetracycline and Ciprofloxacin towards *E. coli* and *E. faecalis*, and the selected sub-MIC wells (yellow) for the subsequent detection (screening antibiotics) by probe **TCF-OTf**.

mg/L	500	250	125	62.5	31.3	15.6	7.8	3.9	1.95	0.98
Streptomycin towards *E. coli*	0.823	0.747	0.587	0.539	0.476	0.389	0.4	0.43	1.998	1.873
	0.834	0.667	0.514	0.484	0.507	0.478	0.528	0.517	2.699	2.106
	0.88	0.747	0.57	0.53	0.526	0.533	0.527	0.779	1.566	2.114
Streptomycin towards *E. faecalis*	0.515	0.634	1.291	1.422	2.149	2.337	2.38	2.495	2.534	2.569
	0.573	0.746	1.48	1.291	1.994	2.297	2.346	2.418	2.434	2.491
	0.338	0.593	1.435	1.63	2.298	2.547	2.533	2.528	2.483	2.537
mg/L	**500**	**250**	**125**	**62.5**	**31.3**	**15.6**	**7.8**	**3.9**	**1.95**	**0.98**
Amikacin towards *E. coli*	0.174	0.82	0.517	0.427	0.439	0.494	0.548	0.536	0.508	1.194
	0.194	0.918	0.566	0.478	0.413	0.44	0.475	0.502	0.49	1.217
	0.234	0.808	0.512	0.426	0.469	0.453	0.428	0.484	0.475	1.064
Amikacin towards *E. faecalis*	0.547	1.438	3.121	3.152	3.056	2.941	2.899	2.865	2.85	2.768
	0.533	1.473	3.209	3.206	3.108	3.059	2.988	2.924	2.852	2.757
	0.322	2.327	3.308	3.289	3.227	3.251	3.245	3.221	3.102	3.055

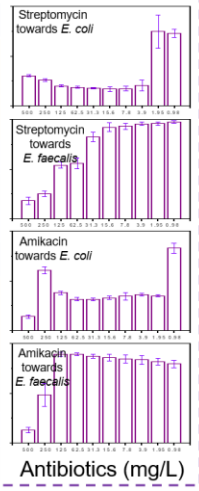


Figure S15. The plate reader detection of OD_{600} absorption intensities of the MIC well plates and the corresponding bar charts in the MIC trial of Streptomycin and Amikacin towards *E. coli* and *E. faecalis*, and the selected sub-MIC wells (yellow) for the subsequent detection (screening antibiotics) by probe **TCF-OTf**.

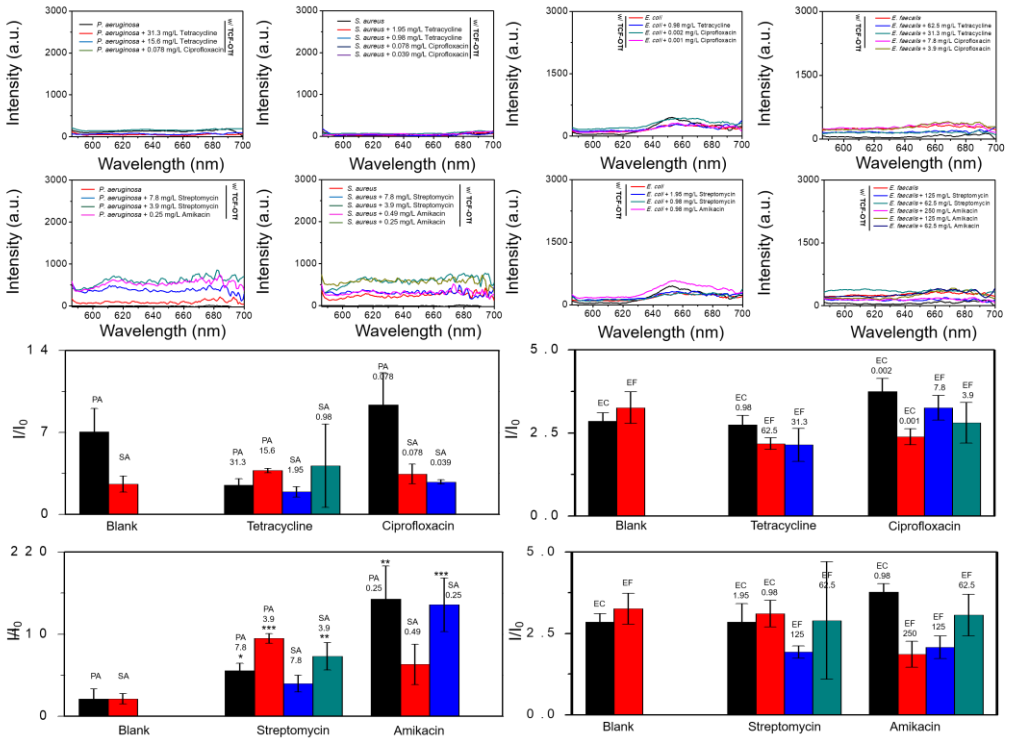


Figure S16. The screening of antibiotics Tetracycline, Ciprofloxacin, Streptomycin, and Amikacin, and corresponding change in fluorescence in detecting $O_2^{\cdot-}$ stress among the sub-MIC wells of *P. aeruginosa* (PA), *S. aureus* (SA), *E. coli* (EC), and *E. faecalis* (EF) using **TCF-OTf**. Unit: mg/L. *P<0.04, **P<0.02, ***P<0.01. λ_{ex} = 560 nm, λ_{em} = 606 nm.

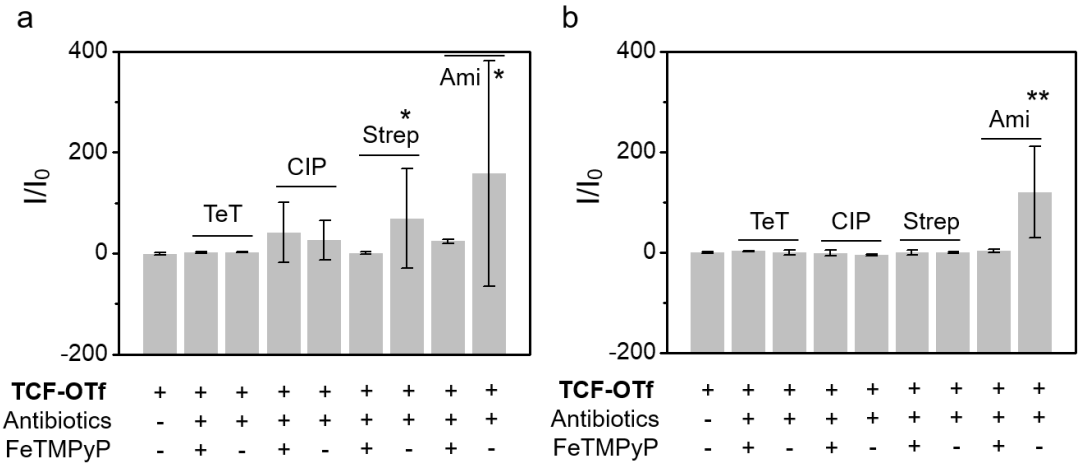


Figure S17. The corresponding change in fluorescence of **TCF-OTf** treated *P. aeruginosa* (a) and *S. aureus* (b) with treatment of sub-MIC concentrations of Tetracycline (TeT), Ciprofloxacin (CIP), Streptomycin (Strep), and Amikacin (Ami), with or without presence of FeTMPyP. *P<0.5, **P<0.15. λ_{ex} = 560 nm, λ_{em} = 606 nm.

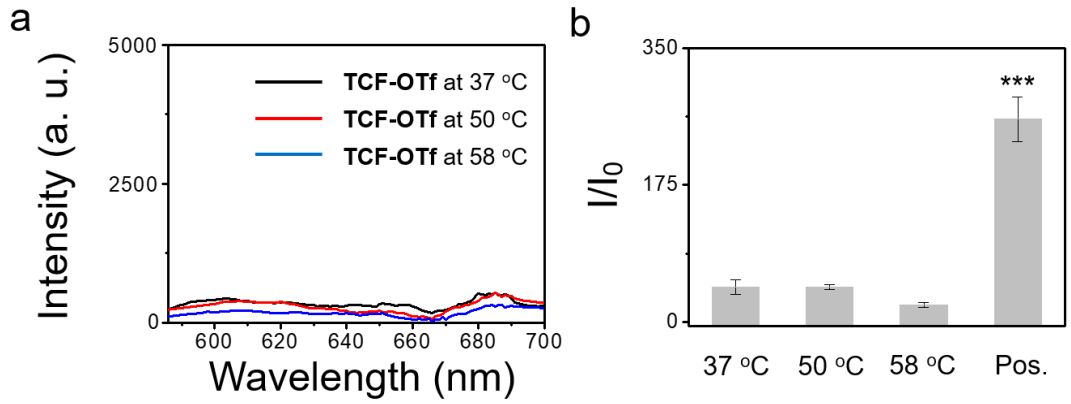


Figure S18. Fluorescence spectra of **TCF-OTf** (10 μM) (a) and corresponding change in fluorescence (b) under different temperatures, indicating its stability. Positive control was used (**TCF-OTf** + $O_2^{\cdot-}$) to show the probe is still active. ***P<0.001. λ_{ex} = 560 nm, λ_{em} = 606 nm.

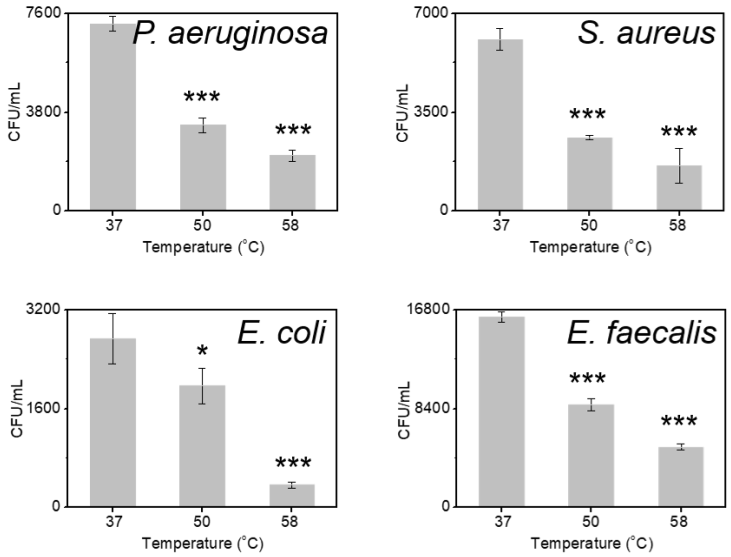


Figure S19. Survival of *P. aeruginosa*, *S. aureus*, *E. coli*, and *E. faecalis* after 1 h heating under 50 °C and 58 °C respectively, compared with that grown in normal conditions (37 °C). *P<0.1, ***P<0.001.

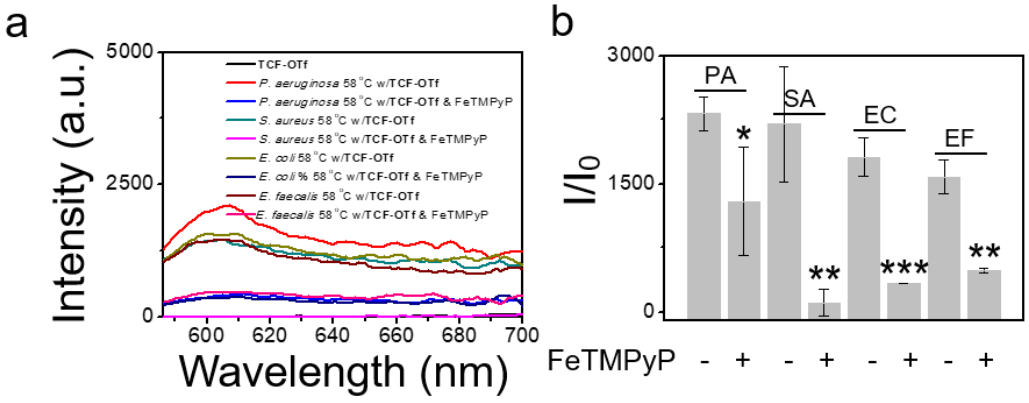


Figure S20. TCF-OTf based fluorescence emission spectrum detection and corresponding change in fluorescence with presence of *P. aeruginosa* (PA), *S. aureus* (SA), *E. coli* (EC), and *E. faecalis* (EF) after 1 h heating under 58 °C, and with or without the further treatment of FeTMPyP. *P<0.1, **P<0.02, ***P<0.001. λ_{ex} = 560 nm, λ_{em} = 606 nm.

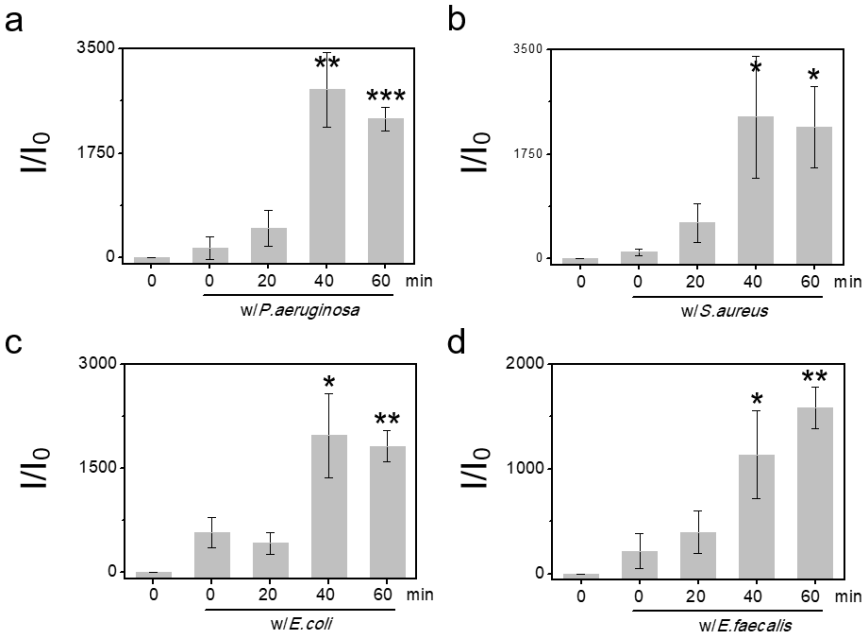


Figure S21. Corresponding change in fluorescence in the **TCF-OTf** based fluorescence emission spectrum detection with presence of *P. aeruginosa* (a), *S. aureus* (b), *E. coli* (c), and *E. faecalis* (d), after 0 – 60 min heating under 58 °C. *P<0.05, **P<0.01, ***P<0.001. λ_{ex} = 560 nm, λ_{em} = 606 nm.

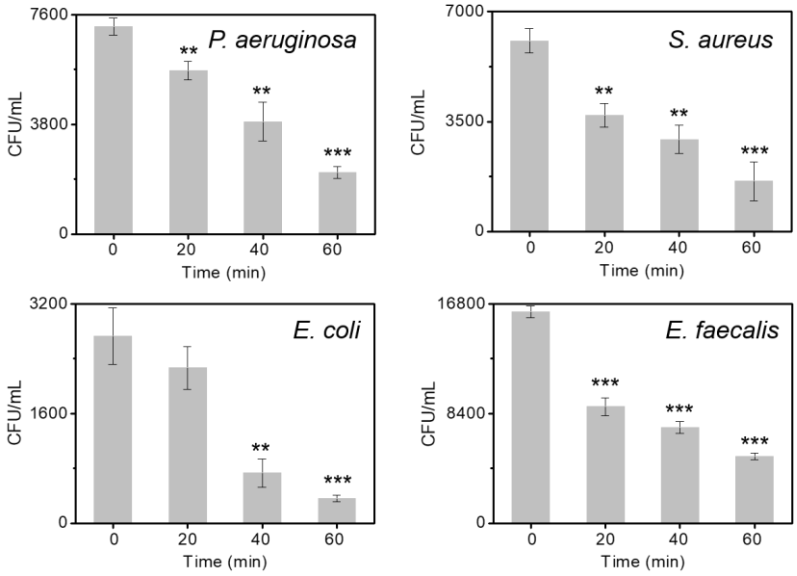


Figure S22. Survival of *P. aeruginosa*, *S. aureus*, *E. coli*, and *E. faecalis* after 0 – 60 min heating under 58 ºC. **P<0.01, ***P<0.001.

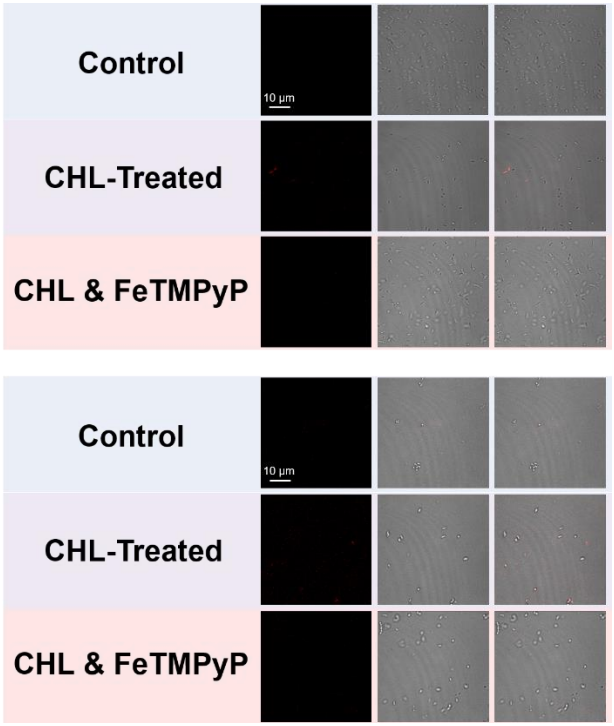


Figure S23. Confocal Laser-Scanning Microscopy (CLSM) imaging of the intracellular signal of **TCF-OTf**-stained *P. aeruginosa* (above) and *E. faecalis* (below) (control), and that with treatment of sub-MIC concentrations of Chloramphenicol (CHL) (31.3 mg/L for *P. aeruginosa*, 125 mg/L for *E. faecalis*), without or with subsequent treatment of FeTMPyP. λ_{ex} = 561 nm (laser source), λ_{em} = 606 nm.

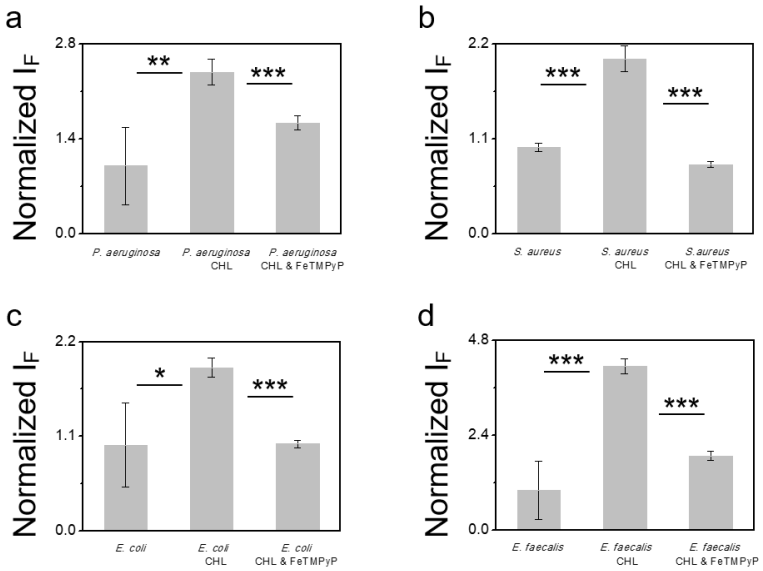


Figure S24. Normalized fluorescence intensities of the Confocal Laser-Scanning Microscopy (CLSM) images of the **TCF-OTf** stained *P. aeruginosa*, *S. aureus*, *E. coli*, and *E. faecalis*, and *P. aeruginosa*, *S. aureus*, *E. coli*, and *E. faecalis* with treatment of sub-MIC concentrations of Chloramphenicol (CHL) (31.3 mg/L for *P. aeruginosa*, 15.6 mg/L for *S. aureus*, 1.95 mg/L for *E. coli*, and 125 mg/L for *E. faecalis*), without or with subsequent treatment of FeTMPyP. This is the quantitative data of Figure 4 and Figure S23. *P<0.03, **P<0.01, ***P<0.001. λ_{ex} = 561 nm (laser source), λ_{em} = 606 nm.

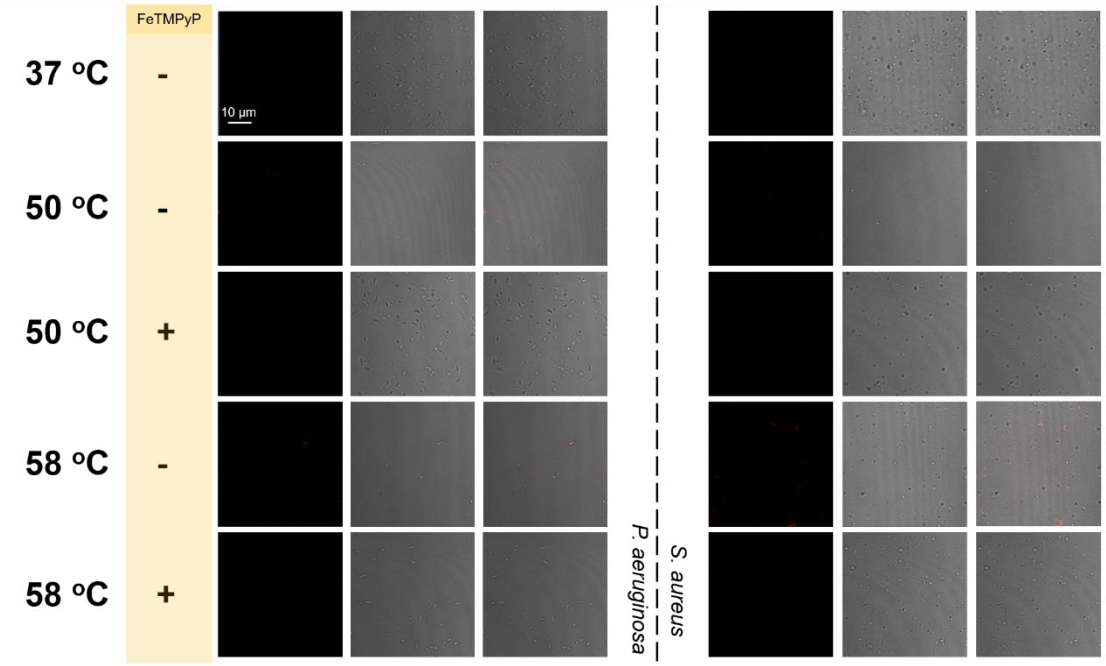


Figure S25. Confocal Laser-Scanning Microscopy (CLSM) imaging of the intracellular signal of **TCF-OTf** stained *P. aeruginosa* and *S. aureus* grown in different temperatures (37°C normal conditions, 50 °C, and 58 °C) for 1 h, without (-) or with (+) subsequent treatment of FeTMPyP. λ_{ex} = 561 nm (laser source), λ_{em} = 606 nm.

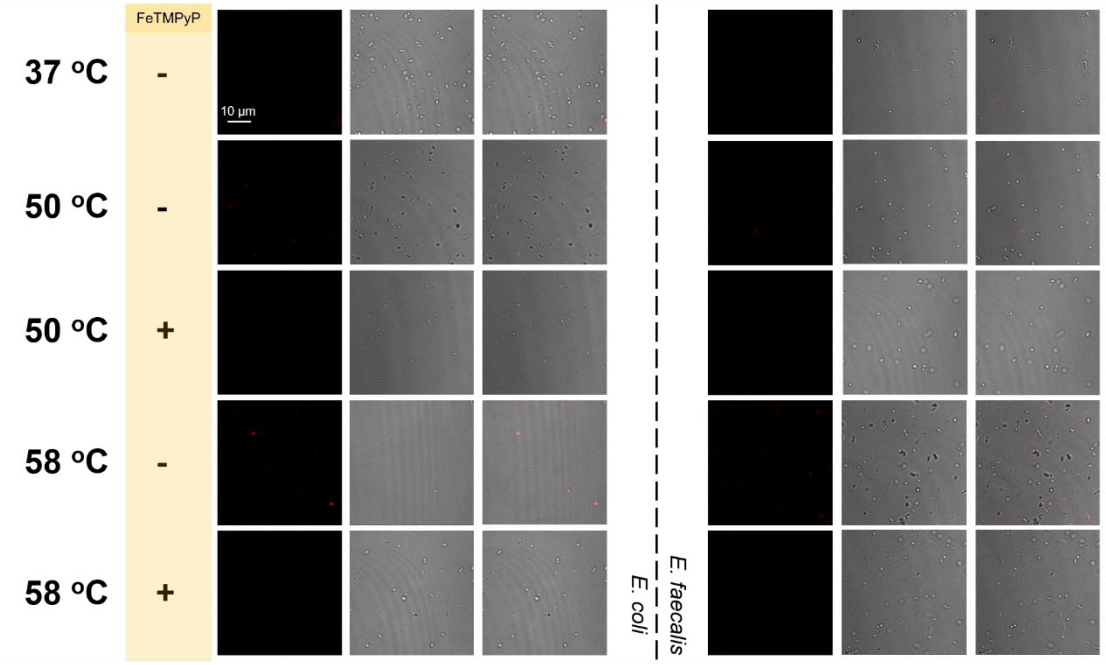


Figure S26. Confocal Laser-Scanning Microscopy (CLSM) imaging of the intracellular signal of **TCF-OTf** stained *E. coli*, and *E. faecalis* grown in different temperatures (37°C normal conditions, 50 °C, and 58 °C) for 1 h, without (-) or with (+) subsequent treatment of FeTMPyP. λ_{ex} = 561 nm (laser source), λ_{em} = 606 nm.

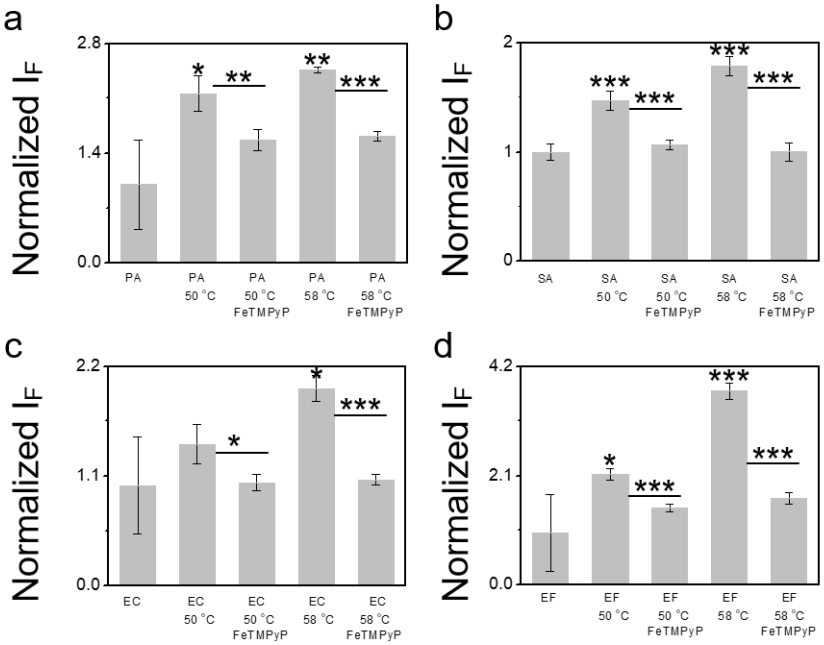


Figure S27. Normalized fluorescence intensities of the Confocal Laser-Scanning Microscopy (CLSM) images of the **TCF-OTf** stained *P. aeruginosa* (PA), *S. aureus* (SA), *E. coli* (EC), and *E. faecalis* (EF) grown in different temperatures (37°C normal conditions, 50 °C, and 58 °C) for 1 h, without (-) or with (+) subsequent treatment of FeTMPyP. This is the quantitative data of Figure S22 and S23. *P<0.1, **P<0.01, ***P<0.001. λ_{ex} = 561 nm (laser source), λ_{em} = 606 nm.

3. Spectra

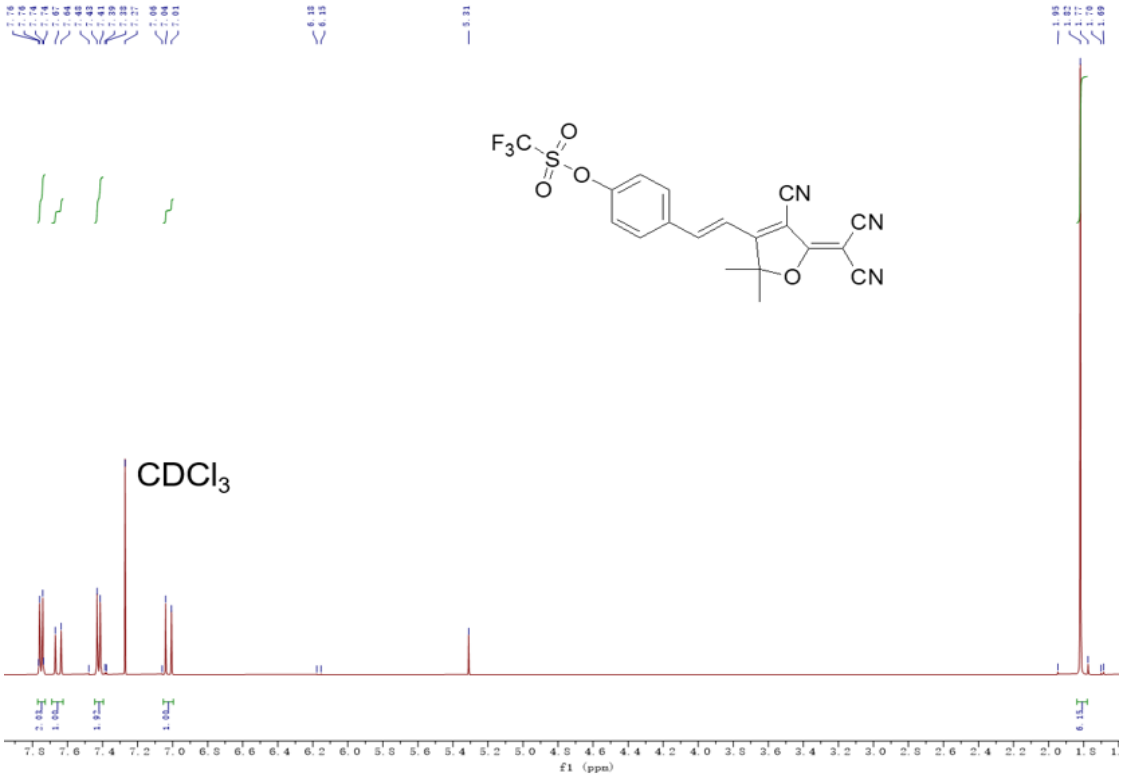


Figure S28. ^{1}H NMR of **TCF-OTf**.

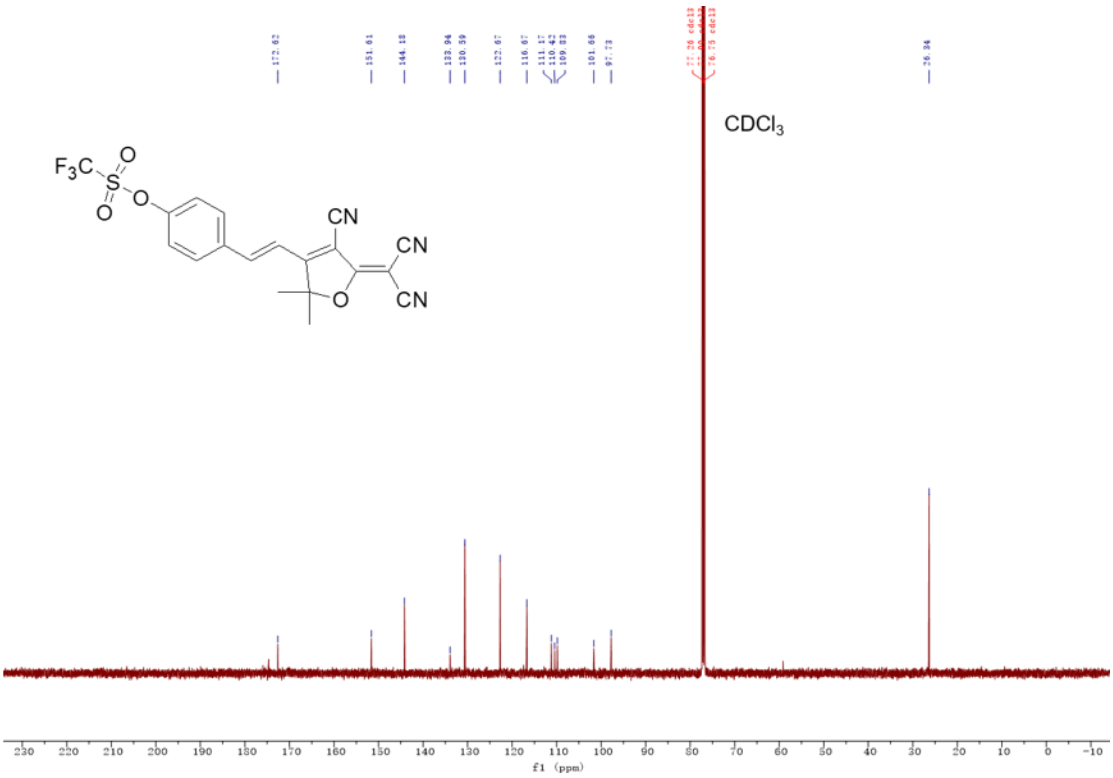


Figure S29. ^{13}C NMR of **TCF-OTf**.

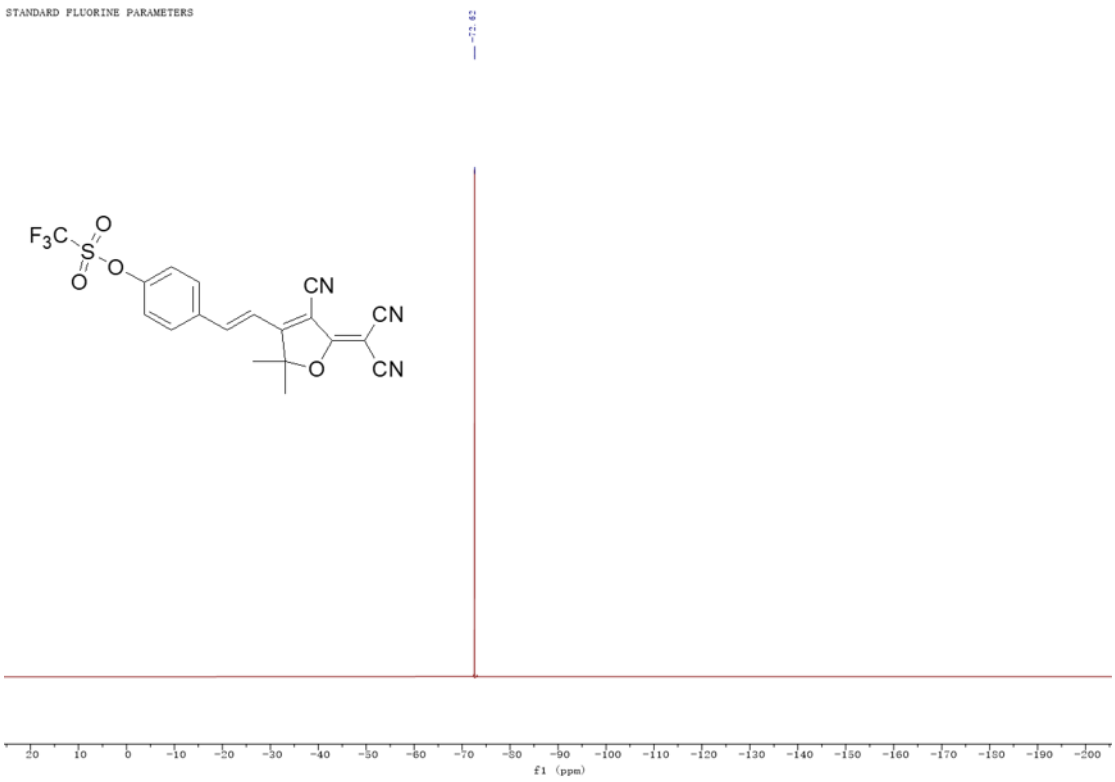


Figure S30. ^{19}F NMR of **TCF-OTf**.

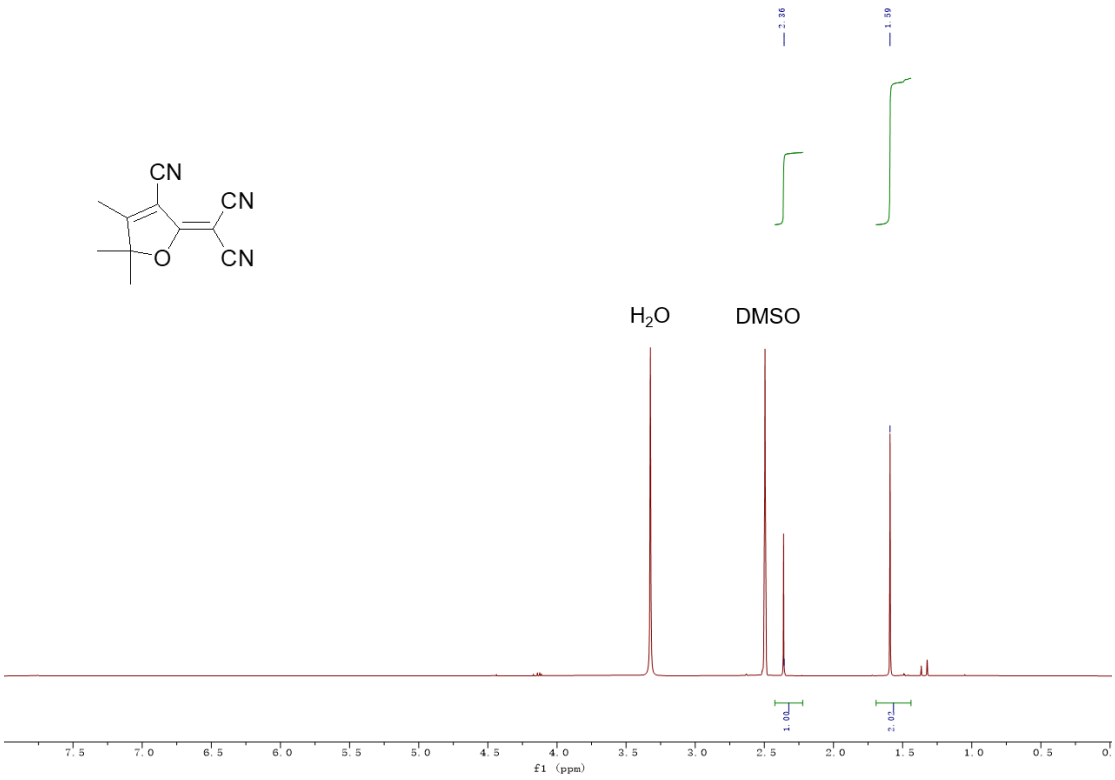


Figure S31. ^{1}H NMR of **TCF**.

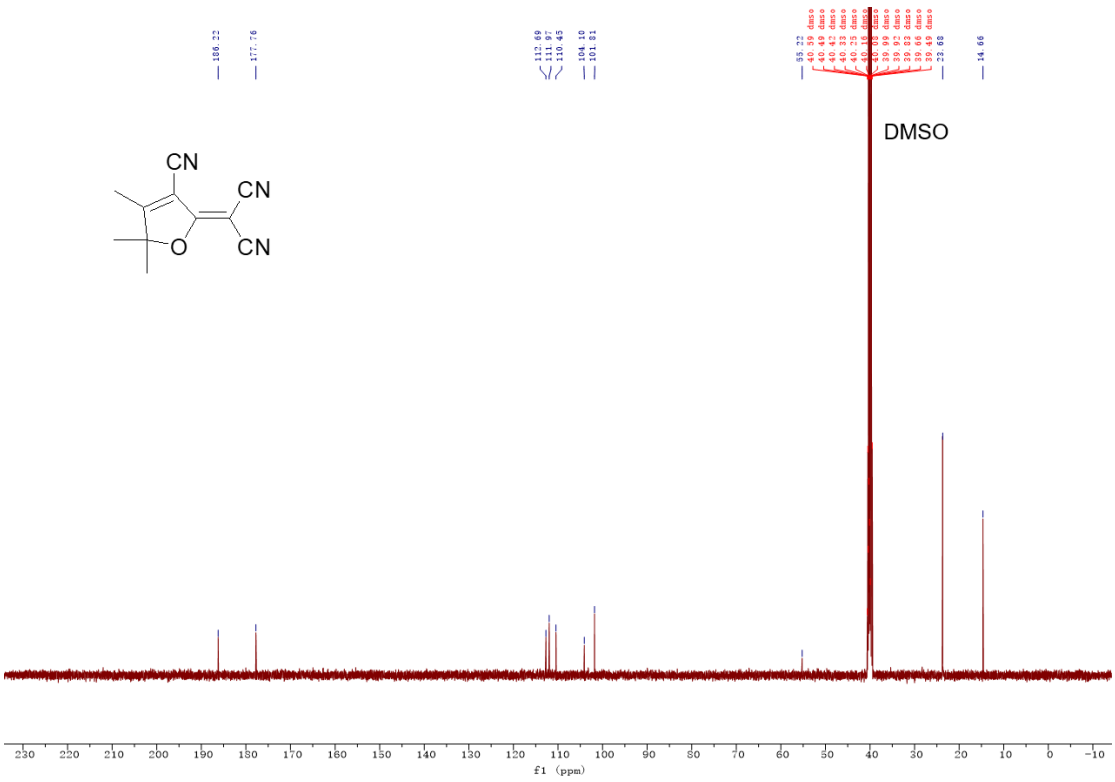


Figure S32. ^{13}C NMR of **TCF**.

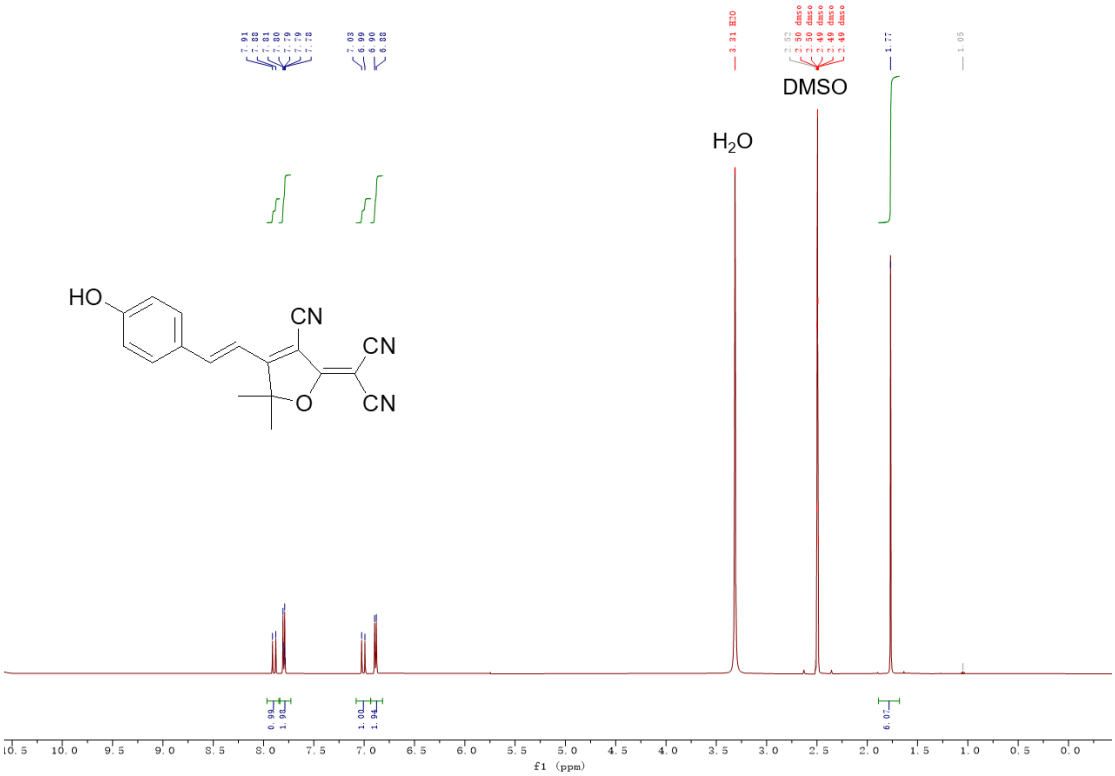


Figure S33. ^{1}H NMR of **TCF-OH**.

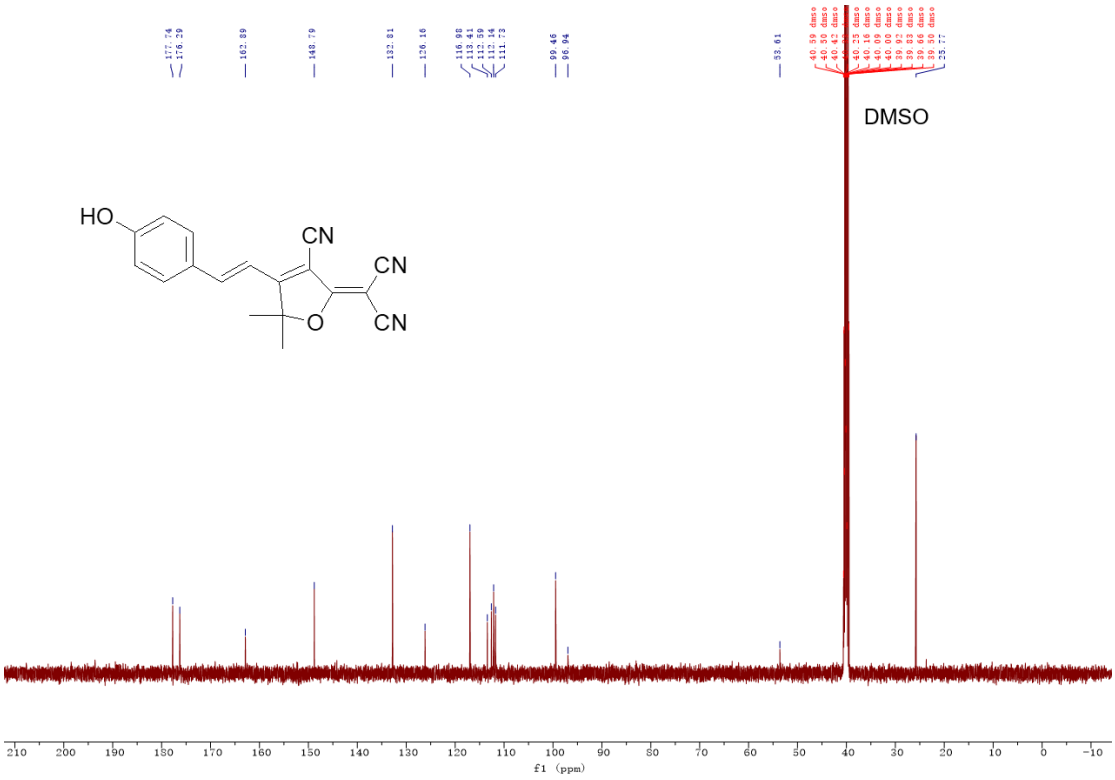


Figure S34. ^{13}C NMR of **TCF-OH**.

4. References

1. A. C. Sedgwick, H.-H. Han, J. E. Gardiner, S. D. Bull, X.-P. He, T. D. James. *Chem. Commun.*, **2017**, *53*, 12822.
2. J. J. Hu, N.-K. Wong, S. Ye, X. Chen, M.-Y. Lu, A. Q. Zhao, Y. Guo, A. C.-H. Ma, A. Y.-H. Leung, J. Shen, D. Yang. *J. Am. Chem. Soc.,* **2015**, *137,* 6837.