Electronic Supporting Information

for

Recognition and Separation of Gram-positive and Gram-negative Bacteria using Polythiophene Derivatives with Phosphonium Groups

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Experimental

Materials

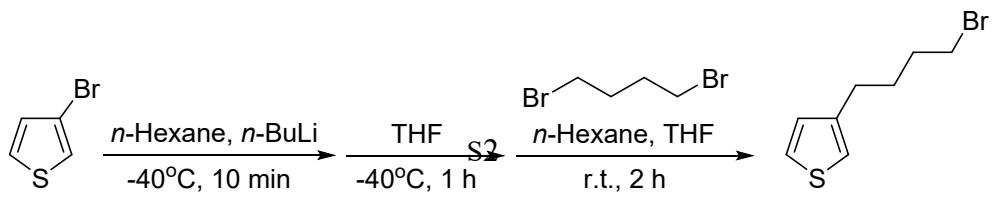
The reagents used in experiments and their purification methods are listed below. 3-Bromothiophene (97%) and 1,4-dibromobutane (95%) were purchased from Tokyo Chemical Industry Co., Ltd. and used after vacuum distillation. The reagents shown below were used as received. *n*-BuLi (2.6 M solution in *n*-hexane) was purchased from Kanto Chemical Co., Ltd. *N*-Bromosuccinimide (NBS), *N,N*-dimethylformamide (DMF), and ^{i}PrMgCl-LiCl (1.3 M in THF) were purchased from FUJIFILM Wako Pure Chemical Industries, Ltd. [1,3-Bis(diphenylphosphino)propane]dichloro nickel (II) (Ni(dppp)Cl_2) was obtained from Sigma-Aldrich.

Measurements for synthesis and identification of polymers

Gas chromatography mass spectrometry (GC-MS) measurements were performed using GCMS-QP2010 SE (SHIMADZU). ^{1}H NMR (400 MHz) measurements were performed on an AscendTM 400 (BRUKER). Chloroform-d_1 ($CDCl_3$), dichloromethane-d_2 (CD_2Cl_2), or methanol-d_4 (CD_3OD) was used as the solvent, and tetramethylsilane (TMS) was used as an internal standard. Elemental analysis was performed using a PE2400-II instrument (PerkinElmer, Inc.) at 975°C. Gel permeation chromatography (GPC) measurements were carried out using an HLC-8320GPC (TOSOH) equipped with a SuperMultiporeHZ-M column (TOSOH). GPC measurements were carried out at 40°C using tetrahydrofuran (THF) as an eluent at a flow rate of 0.35 mL min^{-1}. Polystyrene standards were used. Ultraviolet-visible (UV-vis) absorption and fluorescence spectra were measured using a disposable cell (BrandTech) with an optical path length of 1.0 cm by a V-770 (JASCO) and F-7100 (HITACHI).

Synthesis of monomer

Synthesis of 3-(4-bomobutyl) thiophene (BBT).



3-BT **BBT**

Scheme S1. Synthesis of **BBT**.

BBT was synthesized according to Scheme S1. 3-Bromothiophene, 3-BT, and hexane were mixed in a 100 mL three-necked round-bottom flask in an Ar-filled glove box. *n*-BuLi was added to the solution via a syringe and stirred at −40°C for 10 min. THF was added to the flask and the solution was stirred for 1 h. The temperature was raised to −10°C and 1,4-dibromobutane was added dropwise to the flask. Next, the temperature was raised to room temperature, and the mixture was stirred for 2 h. The mixture was poured into a beaker containing hexane, then methanol was added dropwise to inactivate the remaining BuLi, and 1 M HCl aq. was also added for quenching. The obtained solution was extracted using ethyl acetate, then washed with deionized water and brine solution. After drying by anhydrous magnesium sulfate, the solvent was removed using an evaporator. The resulting yellow transparent liquid was purified by column chromatography (Wakogel FC-40) using *n*-hexane as a solvent (R_f = 0.25).

Table S1. Synthesis condition and yield of **BBT**.

3-BT / g (mmol)	1,4-Dibromobutane / g (mmol)	*n*-BuLi [a] / mL (mmol)	*n*-Hexane / mL	THF / mL	Yield [b] / g (%)
5.22 (32.0)	27.6 (128)	12.3 (32.0)	50.0	5.0	2.88 (41)

[a] 2.6 M solution in *n*-hexane.

[b] Purified by column chromatography (Wakogel FC-40) using *n*-hexane as an eluent.

BBT was identified by ^{1}H NMR and elemental analysis.

^{1}H NMR (400 MHz, CD_3OD); δ 7.27 (q, J = 3.1 Hz, 1H), 6.94 (t, J = 1.6 Hz, 2H), 3.41 (t, J = 6.4 Hz, 2H), 2.63 (t, J = 7.5 Hz, 2H), 1.83 (q, J = 7.5 Hz, 2H), 1.63 (q, J = 7.2 Hz, 2H).

Anal. Calcd. for **BBT**: C, 43.85%; H, 5.06%; S, 14.63%; Br, 36.46%. Found: C, 43.64%; H, 4.80%; S, 14.36%; Br, 33.12%.

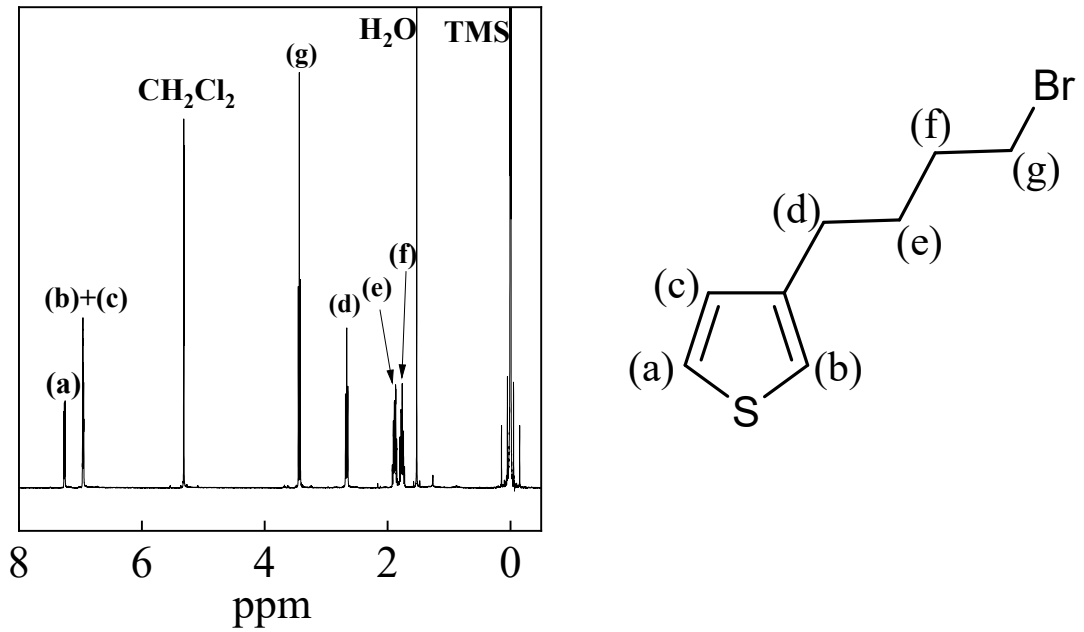
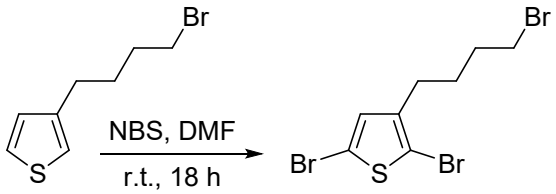


Figure S1. ^{1}H NMR spectrum of **BBT** in CD_2Cl_2.

Synthesis of 2,5-dibromo-3-(4-bromobutyl) thiophene (DBrBBT).



BBT **DBrBBT**

Scheme S2. Synthesis of **DBrBBT**.

DBrBBT was synthesized according to Scheme S2. **BBT** was dissolved in DMF in a 50 mL three-necked round-bottom flask and then DMF was added. While the solution was cooled in an ice bath under N_2 atmosphere, a DMF solution containing NBS was added over 30 min. The mixture was then stirred at room temperature for 18 h. The resulting solution was poured into water to stop the reaction. After extraction with n-hexane, the organic solution was washed with water and brine solution and dried overnight in $MgSO_4$. The yellow liquid was purified by column chromatography (Wakogel FC-40) using *n*-hexane as a solvent (R_f = 0.50).

Table S2. Synthesis condition and yield of **DBrBBT**.

BBT / g (mmol)	NBS / g (mmol)	DMF / mL (mmol)	Yield [a] / g (%)
2.80 (12.8)	4.78 (26.9)	19.0 (274)	3.90 (81)

[a] Purified by column chromatography (Wakogel FC-40) using *n*-hexane as an eluent.

DBrBBT was identified by ^{1}H NMR and elemental analysis.

^{1}H NMR (400 MHz, CD_3OD); δ 6.91 (s, 1H), 3.42 (t, J = 4.4 Hz, 2H), 2.55 (t, J = 4.8 Hz, 2H), 1.84 (q, J = 7.4 Hz, 2H), 1.58 (q, J = 7.6 Hz, 2H).

Anal. Calcd. for (**DBrBBT**): C, 25.49%; H, 2.49%; S, 8.51%; Br, 63.60%. Found: C, 25.90%; H, 2.08%; S, 8.67%; Br, 63.04%.

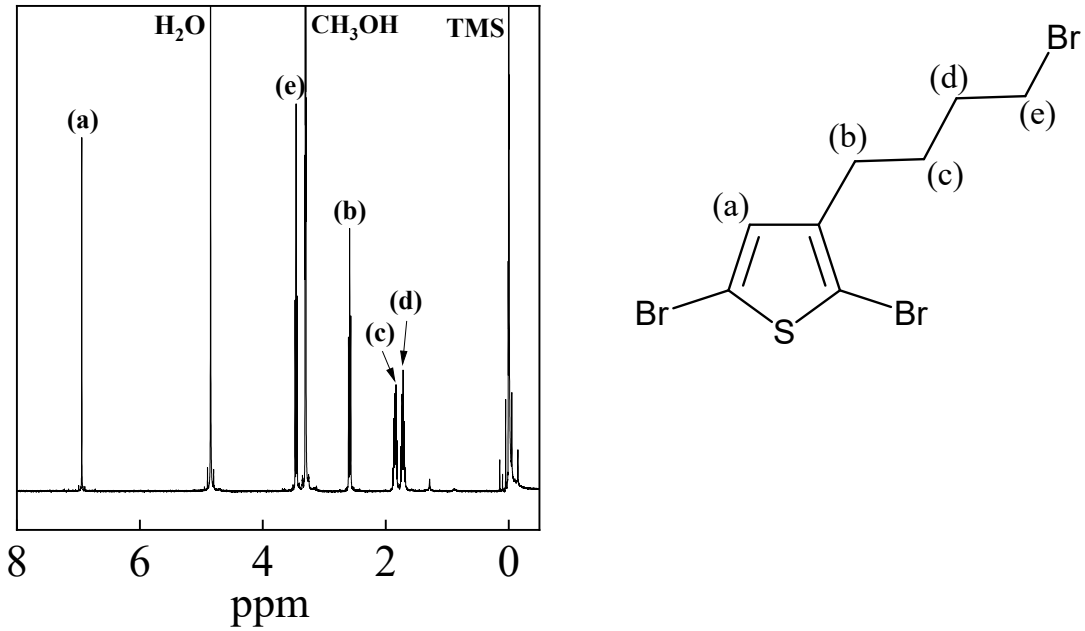
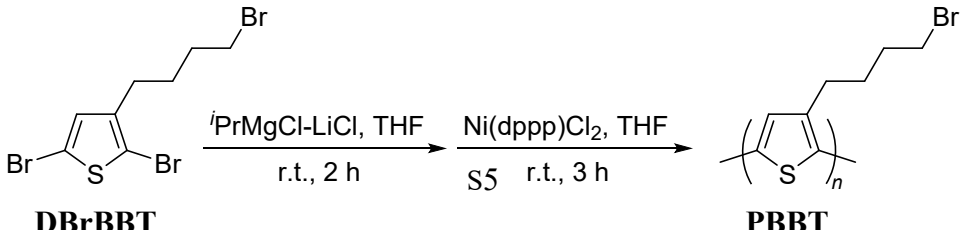


Figure S2. ^{1}H NMR spectrum of **DBrBBT** in CD_3OD.

Synthesis of polymers

Synthesis of poly[3-(4-bromobutyl) thiophene] (PBBT).



DBrBBT **PBBT**

Scheme S3. Synthesis of **PBBT**.

PBBT was synthesized according to Scheme S3. **DBrBBT** was dissolved in THF in a 50 mL eggplant flask in an Ar-filled glove box. ^{i}PrMgCl-LiCl THF solution was added dropwise to the flask using a syringe, followed by reaction for 2 h with stirring at room temperature. The catalyst solution was also prepared by adding 10 mL THF to Ni(dppp)Cl_2. The prepared Ni(dppp)Cl_2 solution was added through a syringe to the Grignard monomer solution and stirred at room temperature for 3 h. The reaction was quenched by pouring the solution into a mixture of 300 mL methanol and 5 mL dilute hydrochloric acid. The crude polymer was obtained as a solid through vacuum filtration. After reprecipitation using chloroform as a good solvent and *n*-hexane as a poor solvent, a reddish purple solid was obtained by suction filtration.

Table S3. Synthesis condition and yield of **PBBT**.

Lot.	**DBrBBT** / g (mmol)	THF / mL	^{i}PrMgCl-LiCl [a] / mL (mmol)	Ni(dppp)Cl_2 / mL (mmol)	[monomer] / [Ni]	Yield [b] / mg (%)
-01	0.35 (0.93)	1.60	0.71 (0.93)	1.55 (2.86)	15	63.0 (31)
-02	2.00 (5.30)	30.0	4.08 (5.30)	12.7 (23.4)	15	273 (23)

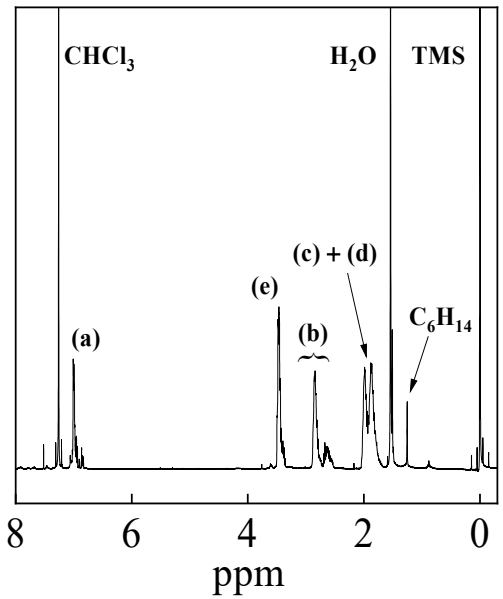
[a] 1.3 M solution in THF.

[b] Purified by reprecipitation using *n*-hexane.

Identification of **PBBT** was performed by GPC and ^{1}H NMR. Number-average molar mass (M_n), weight-average molar mass (M_w), molar mass dispersion (M_w/M_n), and degree of polymerization *n* obtained from GPC using THF as the solvent are listed in Table S4.

Table S4. Molecular weight of **PBBT**.

Lot.	M_n	M_w	M_w/M_n	*n*
PBBT-01	2,448	3,771	1.54	10.8
PBBT-02	2,339	2,974	1.24	10.6



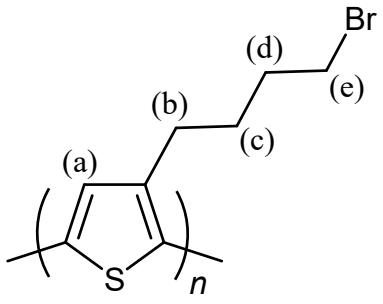
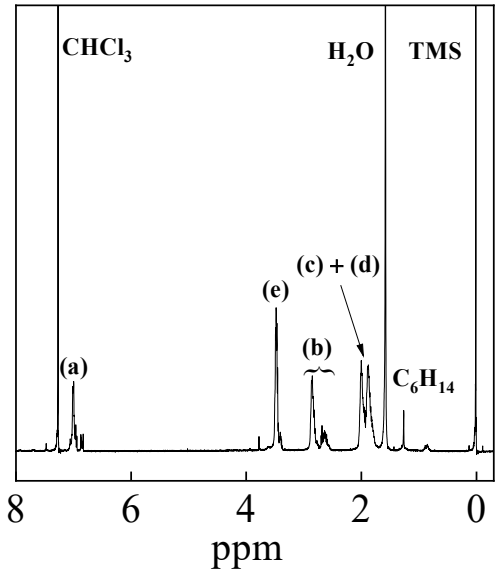
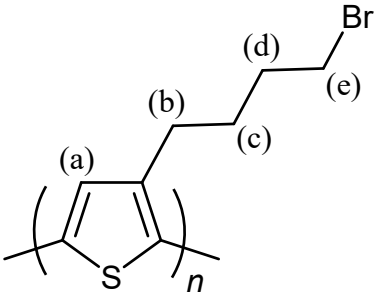


Figure S3. ^{1}H NMR spectrum of **PBBT-01** in $CDCl_3$.



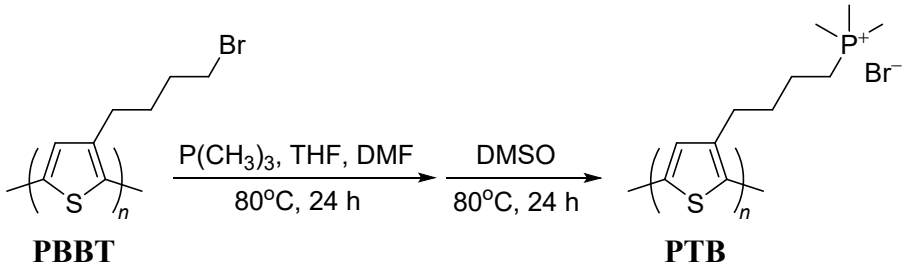


^{1}H NMR (400 MHz, $CDCl_3$); δ 6.98 (s, 1H), 3.47 (t, J = 6.8 Hz, 2H), 2.85-2.53 (t, J = 3.2 Hz, 2H), 1.92 (br, 2H), 1.89 (br, 2H)

Synthesis of phosphonium polymers

By using **PBBT-01** and **-02** as precursor polymers, **PTB**, **Et-PTB**, and **Ph-PTB** were synthesized.

Synthesis of poly[3-(4-trimethylphosphinobutyl) thiophene bromide] (PTB).



PBBT **PTB**

Scheme S4. Synthesis of **PTB**.

PTB was synthesized according to Scheme S4. **PBBT-01** was placed in a 20 mL three-necked round-bottom flask, and 2.0 mL of THF and 3.0 mL of DMF were added. After adding 1.0 mL of $P(Me)_3$ THF solution to the flask, the mixture was heated and stirred under N_2 atmosphere at 80°C for 24 h. To this was added 3.0 mL of DMSO and the mixture was heated and stirred at the same temperature for another 24 h. The resulting reaction solution was poured into 150 mL of diethyl ether. After suction filtration, a purple solid was obtained. A cast film was collected by dissolving the solid in a small amount of methanol and cast on a Teflon sheet.

Table 4. Synthesis condition and yield of **PTB**.

PBBT [a] / mg (mmol)	$P(Me)_3$ [b] / mL (mmol)	THF / mL	DMF / mL	DMSO / mL	Yield [c] / mg (%)
44.0 (18.0)	1.00 (1.00)	2.00	3.00	3.00	59.0 (97)

[a] **PBBT-01** was used, M_n = 2,448, according to the result of GPC. [b] 1.0 M solution in THF.
[c] Purified by reprecipitation using diethyl ether.

Phosphonium degree of **PTB** was determined by ^{1}H NMR (Figure S5). The integral ratio was (a) : (b) : (c) + (d) : (e) : (f) = 0.99 : 2.00 : 3.81 : 2.00 : 8.58, in agreement with the theoretical value of 1 : 2 : 4 : 2 : 9. The results of this measurement confirmed the synthesis and purification of **PTB**.

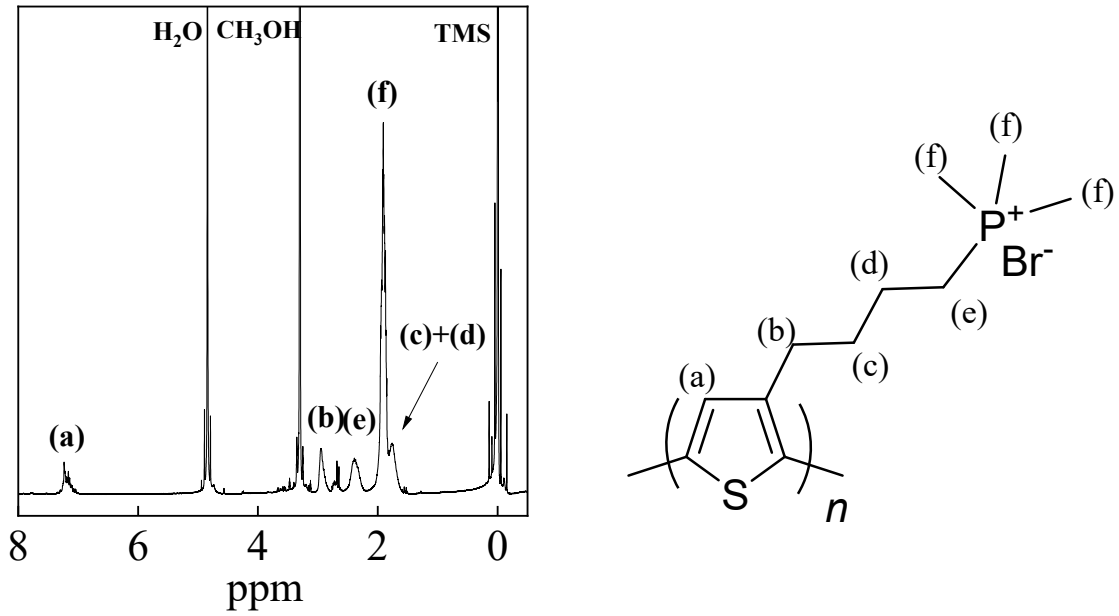


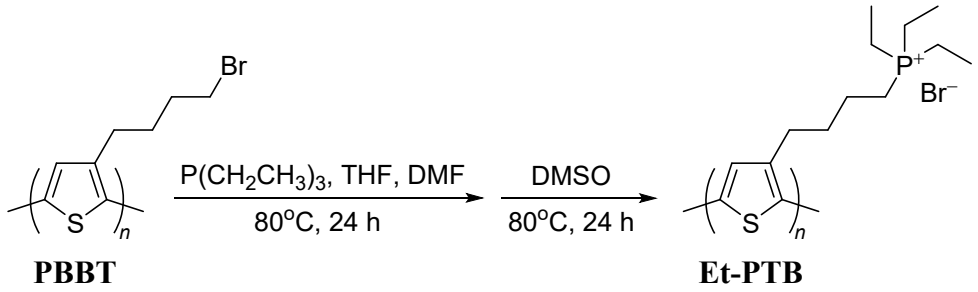
Figure S5. ^{1}H NMR spectrum of **PTB** in CD_3OD.

The integral values of the terminal bromine or α-hydrogen of the phosphonium group were used to calculate the phosphonation ratio. Specifically, the chemical shift derived from e in **PBBT** before phosphonation at 3.42 ppm, and the chemical shift derived from e in **PTB** after phosphonation at 2.26 ppm, were used. The following equation was used in the calculations. The phosphonium conversion calculated from the integral ratio was 97%.

$$\frac{Integral\ value\ of\ 2.26\ ppm}{Integral\ value\ of\ 2.26\ ppm + \ Integral\ value\ of\ 3.42\ ppm} \times 100$$

^{1}H NMR (400 MHz, CD_3OD); δ 7.11 (s, 1H), 2.92 (br, 2H), 2.32 (br, 2H), 1.88 (br, 9H), 1.76 (br, 4H).

Synthesis of poly[3-(4-triethylphosphinobutyl) thiophene bromide] (Et-PTB)



PBBT **Et-PTB**

Scheme S5. Synthesis of **Et-PTB**.

Et-PTB was synthesized according to Scheme S5, similar to **PTB**, as shown above. A purple solid was finally obtained.

Table S5. Synthesis condition and yield of **Et-PTB**.

PBBT [a] / mg (mmol)	$P(CH_2CH_3)_3$ [b] / mL (mmol)	THF / mL	DMF / mL	DMSO / mL	Yield [c] / mg (%)
53.0 (21.7)	1.30 (1.30)	2.00	3.00	3.00	78.4 (96)

[a] **PBBT-02** was used, M_n = 2,339, according to the result of GPC. [b] 1.0 M solution in THF.
[c] Purified by reprecipitation using diethyl ether.

Similar to **PTB**, the phosphonium degree of **Et-PTB** was determined by ^{1}H NMR (Figure S6). The integral ratio was (a) : (b) : (c) : (d) : (e) + (f) : (g) = 1.01 : 2.12 : 2.00 : 2.12 : 8.00 : 8.97, in agreement with the theoretical value of 1 : 2 : 2 : 2 : 2 : 8 : 9. The results of this measurement confirmed the synthesis and purification of **Et-PTB**. The phosphonium conversion ratio calculated from the integral ratio was 99%.

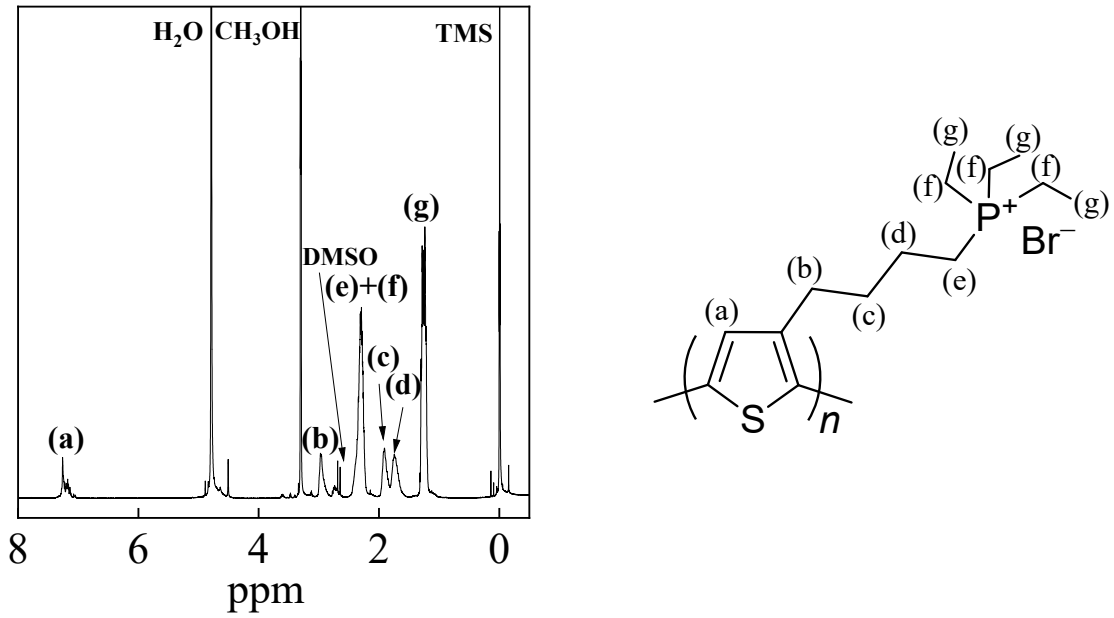
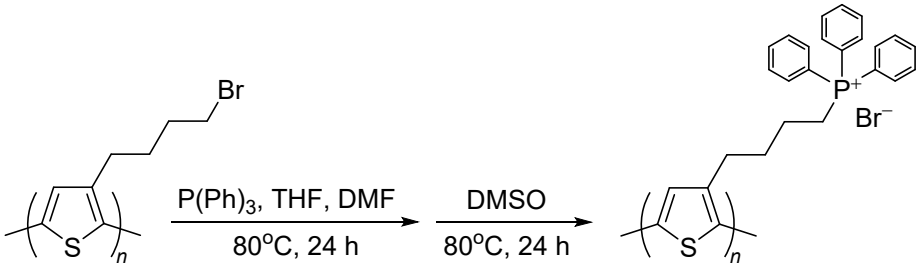


Figure S6. ^{1}H NMR spectrum of **Et-PTB** in CD_3OD.

^{1}H NMR (400 MHz, CD_3OD); δ 7.11 (s, 1H), 2.98 (br, 2H), 2.32 (br, 8H), 1.93 (br, 2H), 1.77 (br, 2H), 1.25 (m, J = 8.0 Hz, 9H).

Synthesis of poly[3-(4-triphenylphosphinobutyl) thiophene bromide] (Ph-PTB).



PBBT **Ph-PTB**

Scheme S6. Synthesis of **Ph-PTB**.

Ph-PTB was synthesized according to Scheme S6, similar to **PTB**, as shown above. A purple solid was finally obtained.

Table S6. Synthesis condition and yield of **Ph-PTB**.

PBBT [a] / mg (mmol)	$P(Ph)_3$ / mg (mmol)	THF / mL	DMF / mL	Yield [b] / mg (%)
50.0 (20.4)	322 (1.23)	3.50	3.00	90.9 (82)

[a] **PBBT-02** was used, M_n = 2,339, according to the result of GPC.

[b] Purified by reprecipitation using diethyl ether.

Similar to **PTB**, the phosphonium degree of **Ph-PTB** was determined by ^{1}H NMR (Figure S7). The identification of **Ph-PTB** was performed by ^{1}H NMR. Figure 7 shows the results of ^{1}H NMR measurements. Chemical shifts attributable to each proton were observed. The integral ratio was (a) : (b) : (c) : (d) : (e) : (f) = 1.37 : 1.87 : 2.00 : 2.79 : 2.70 : 14.91, in agreement with the theoretical value of 1 : 2 : 2 : 2 : 2 : 15. The results of this measurement confirmed the synthesis and purification of **Ph-PTB**. The phosphonium conversion ratio calculated from the integral ratio was 95%.

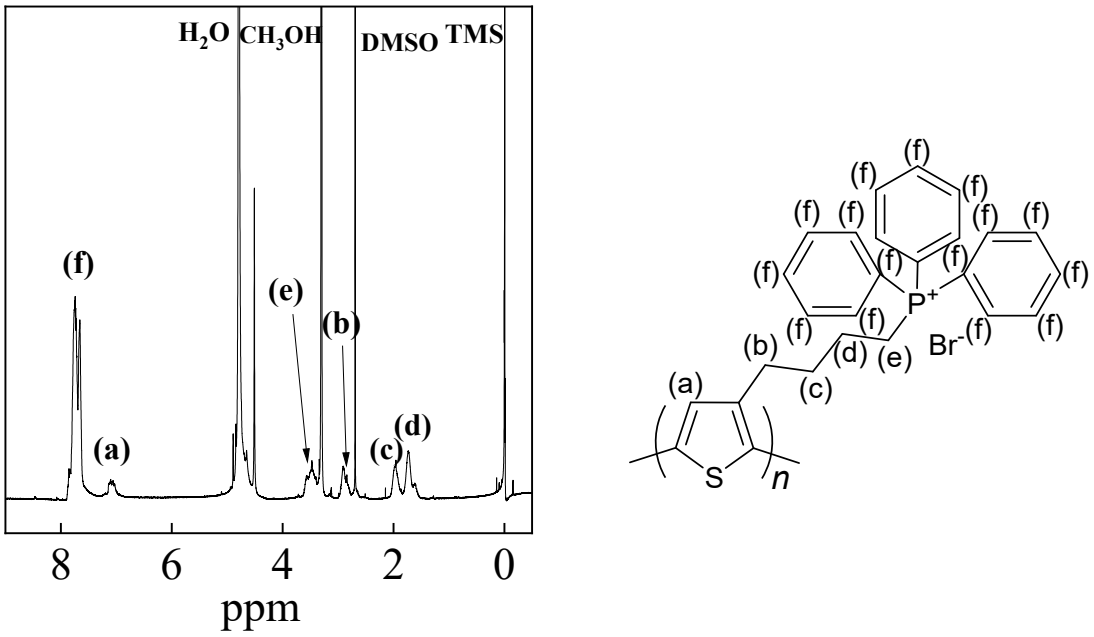


Figure S7. ^{1}H NMR spectrum of **Ph-PTB** in CD_3OD.

^{1}H NMR (400 MHz, CD_3OD); 7.95–7.56 (s, 15H), 7.11 (s, 1H), 3.51 (br, 2H), 2.88 (br, 2H), 1.96 (br, 2H), 1.74 (br, t).

Information on the polythiophene derivatives used in this study is shown in Figure S8 and Table S7.

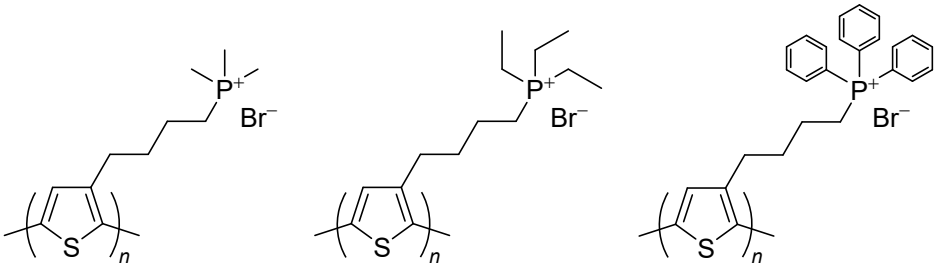


Figure S8. Chemical structures of polythiophene derivatives.

Table S7. Characteristics of polythiophene derivatives.

	M_n [a]	M_w/M_n	n	Introduction ratio of phosphonium groups / %
PTB	2,448	1.54	11	97
Et-PTB	2,339	1.24	11	99
Ph-PTB	2,339	1.24	11	95

[a] M_n of precursor polymer **PBBT.**

Bacterial detection experiment

Preparation of buffer, media, and suspension

Tris-HCl buffer

Trizma® base (0.97 g) and 6.61 g of Trizma® hydrochloride were dissolved in 1000 mL of purified water to prepare 0.05 M Tris-HCl buffer at pH 7.40.

LB liquid medium

Tryptone USP (10 g), 5 g of bactoTM Yeast Extract, and 10 g of sodium chloride were dissolved in 1000 mL of pure water and transferred to a bottle. The solution was sterilized using an autoclave (120°C, 20 min) and used as the LB liquid medium.

LB agar medium

To 1000 mL of LB liquid medium, 15 g of agar was added, and the agar was dissolved by heating and stirring at 60°C. After sterilization using an autoclave (120°C, 20 min), 20 mL was poured into petri dishes and incubated at 37°C overnight in an incubator IC402 (Yamato).

Preparation of bacterial suspension

Staphylococcus aureus (IAM1011) and *Bacillus subtilis* var. *natto* were used as Gram-positive bacteria, while *Escherichia coli* (K12W3110) and *Pseudomonas aeruginosa* (ATCC27853) were used as Gram-negative bacteria. Frozen glycerol stock was scraped off with a disposable loop and applied to LB agar medium for individual culture. This was kept overnight in a 37°C incubator and used as a master plate. A single colony from the master plate was removed using a toothpick, transferred to 5 mL of LB liquid medium in a 50 mL tube, and incubated overnight in a constant temperature shaker incubator BR-42FL (TAITEC) (37°C, 180 rpm). The optical density at 600 nm (OD_{600}) was measured using a UV-visible spectrophotometer UV-12000V (SHIMADZU), and the appropriate concentrations were prepared by diluting the bacterial suspension with LB liquid medium. The following indices were used to prepare the concentrations of the bacterial suspensions.

S. aureus: OD_{600} = 1.0, CFU = 4.5×10^8 cells mL^{-1}

B. subtilis: OD_{600} = 1.0, CFU = 1.0×10^9 cells mL^{-1}

E. coli: OD_{600} = 1.0, CFU = 1.0×10^9 cells mL^{-1}

P. aeruginosa : OD_{600} = 1.0, CFU = 3.7×10^8 cells mL^{-1}

It is widely recognized that the final OD_{600} values may vary depending on the bacterial batch. In the comparative experiments presented within the same graph, an identical batch was used for all measurements, ensuring that the data could be evaluated on a consistent basis.

Measurements

Dynamic light scattering and zeta potential measurements were conducted using an ELSZ-2000ZS (Otsuka Electronics) at 25°C. Bacterial morphology was observed using an Axiovert 200M (Zeiss) fluorescence microscope (λ_{ex} = 550 nm, λ_{em} = 570 nm) with 43 HE DsRED (489043-9901-000). Each solution (15 μL) was dropped onto a glass slide MAS-01 (MATSUNAMI) to make the samples, and fluorescence microscopic observation was performed.

Selectivity for high molecular weight phosphate compounds

To investigate the response of **PTB** to phosphate compounds, adenosine monophosphate (AMP), adenosine diphosphate (ADP), adenosine triphosphate (ATP), random-DNA and RNA (Deoxyribonucleic acid, from salmon sperm was purchased from FUJIFILM Wako Pure Chemical Industries) were used. UV-vis absorption and fluorescence measurements were made when phosphate compounds ($[P^-]$ = 10 μM) were added to a Tris-HCl buffer solution of **PTB** ($[P^+]$ = 10 μM). Ultraviolet-visible (UV-vis) absorption spectroscopy measurements were performed using a V-770 spectrophotometer (JASCO). Measurements were performed using a disposable cell (BrandTech) with a path length of 1.0 cm, under the following conditions: absorbance mode, medium scan speed, and a slit width of 2.0 nm. Fluorescence spectroscopy measurements were performed using a F-7100 (HITACHI). The excitation wavelength was set to the maximum absorption wavelength, and fluorescence spectra were recorded from 5 nm above the excitation wavelength to 800 nm. The scan speed was set to 240 nm min^{-1}, and the photomultiplier tube voltage was set to 400 V.

Separation of bacteria

The centrifuge was run using an LC-200 (TOMY). Separation of *S. aureus* and *E. coli* from coexisting conditions was performed under room temperature conditions at 500 rpm for 3 min.

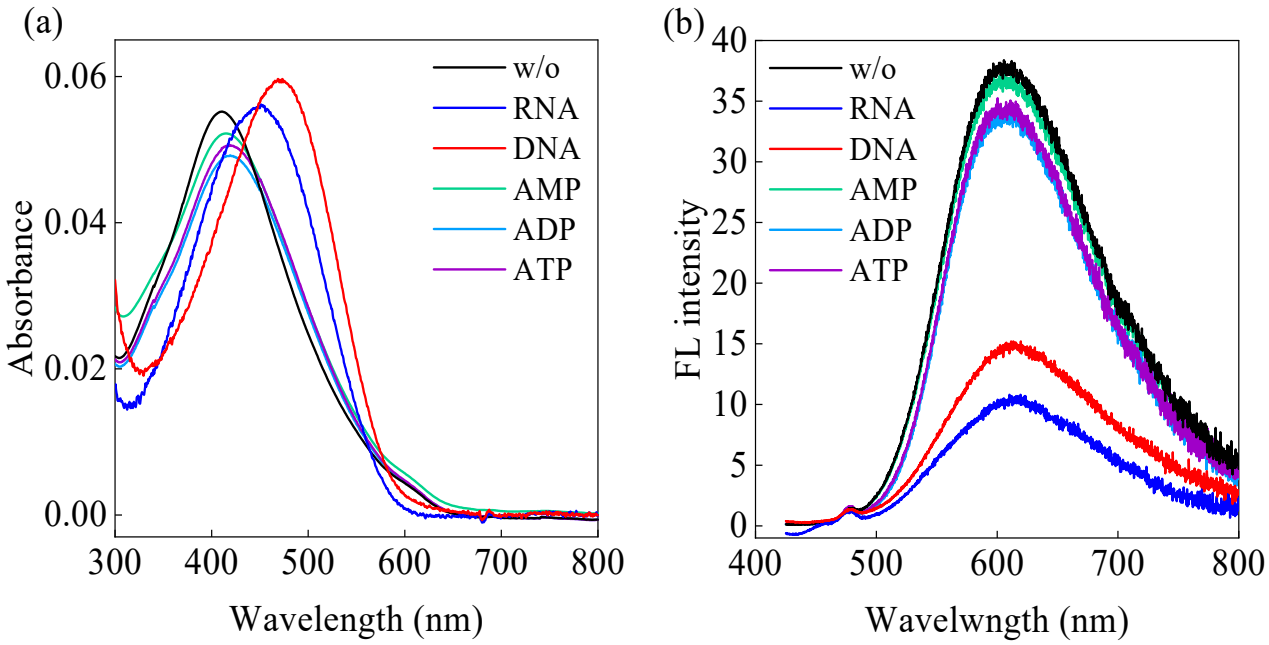


Figure S9. (a) UV-vis absorption and (b) fluorescence (λ_{ex} = 410 nm) spectra of **PTB** in Tris-HCl buffer after addition of random-RNA, DNA, AMP, ADP and ATP. [P^+] = 10 μM. [P^-] = 10 μM.

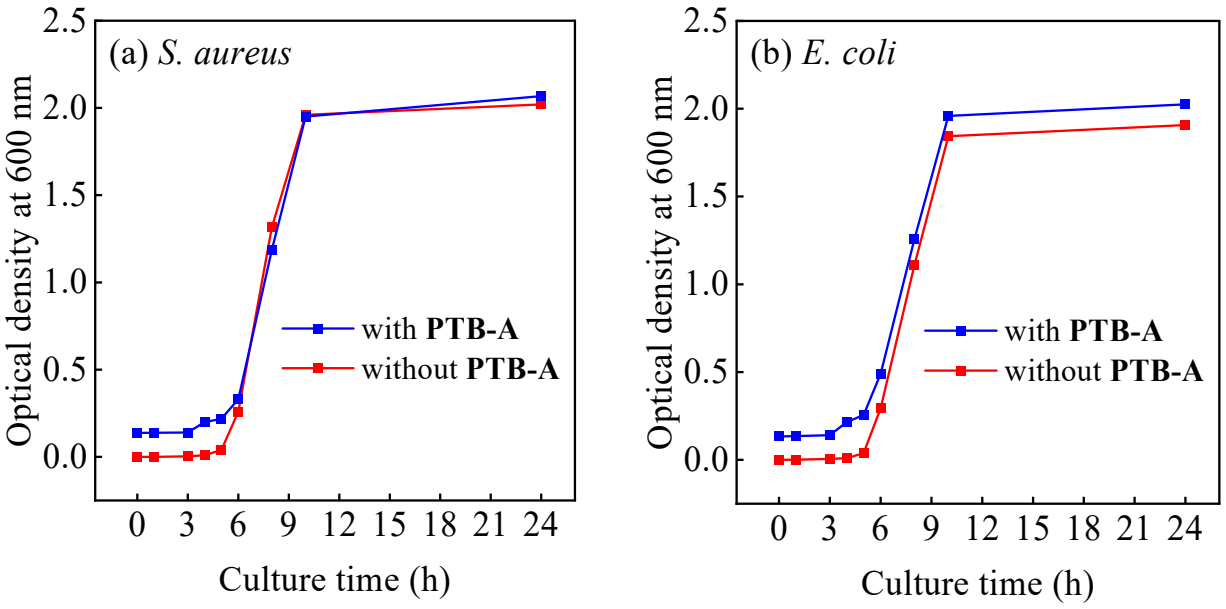


Figure S10. Growth curves of (a) *S. aureus*, and (b) *E. coli* in the absence and presence of **PTB-A** (M_n=2,516, M_w/M_n = 1.24, n = 11.2, introduction ratio of ammonium: 100%).

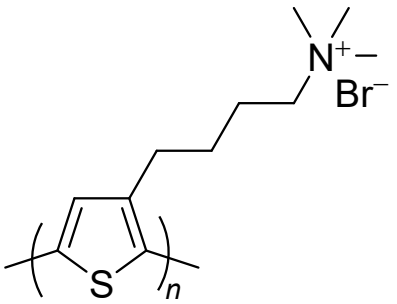


Figure S11. Chemical structure of **PTB-A**.

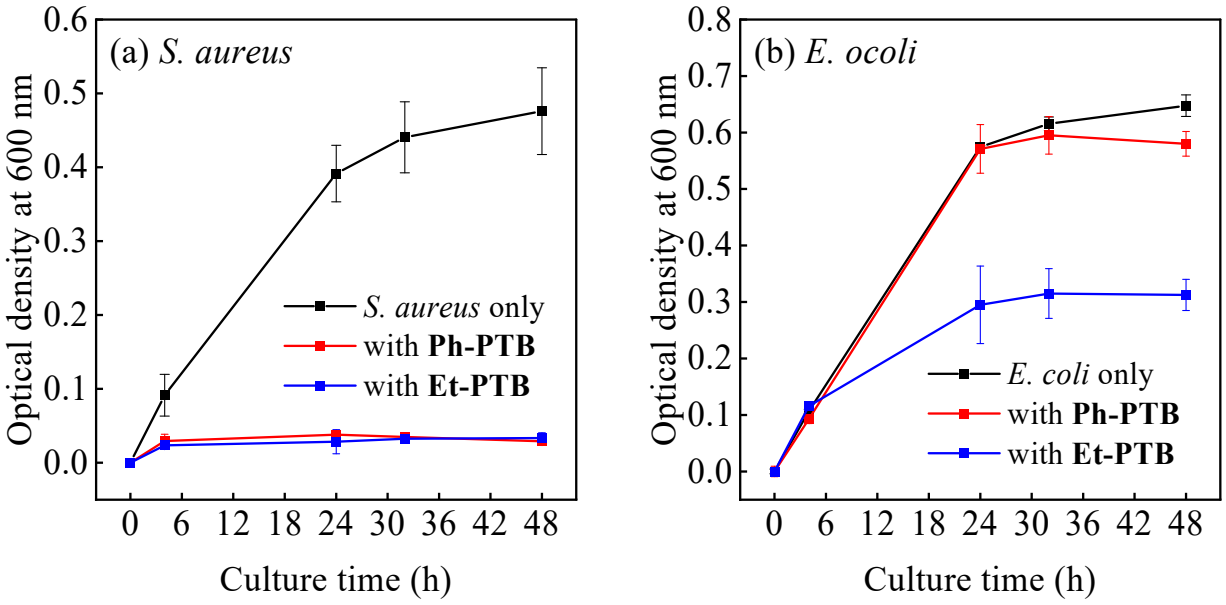


Figure S12. Growth curves of (a) *S. aureus*, and (b) *E. coli* in the absence and presence of **Et-PTB** or **Ph-PTB**.

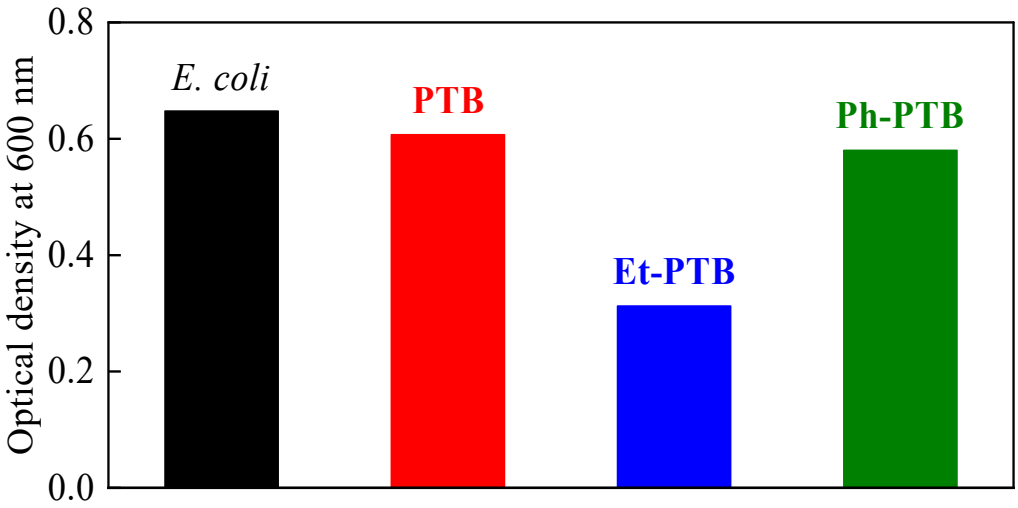


Figure S13. OD_{600} after 24 h culture of *E. coli* with added **PTB**, **Et-PTB**, or **Ph-PTB**.

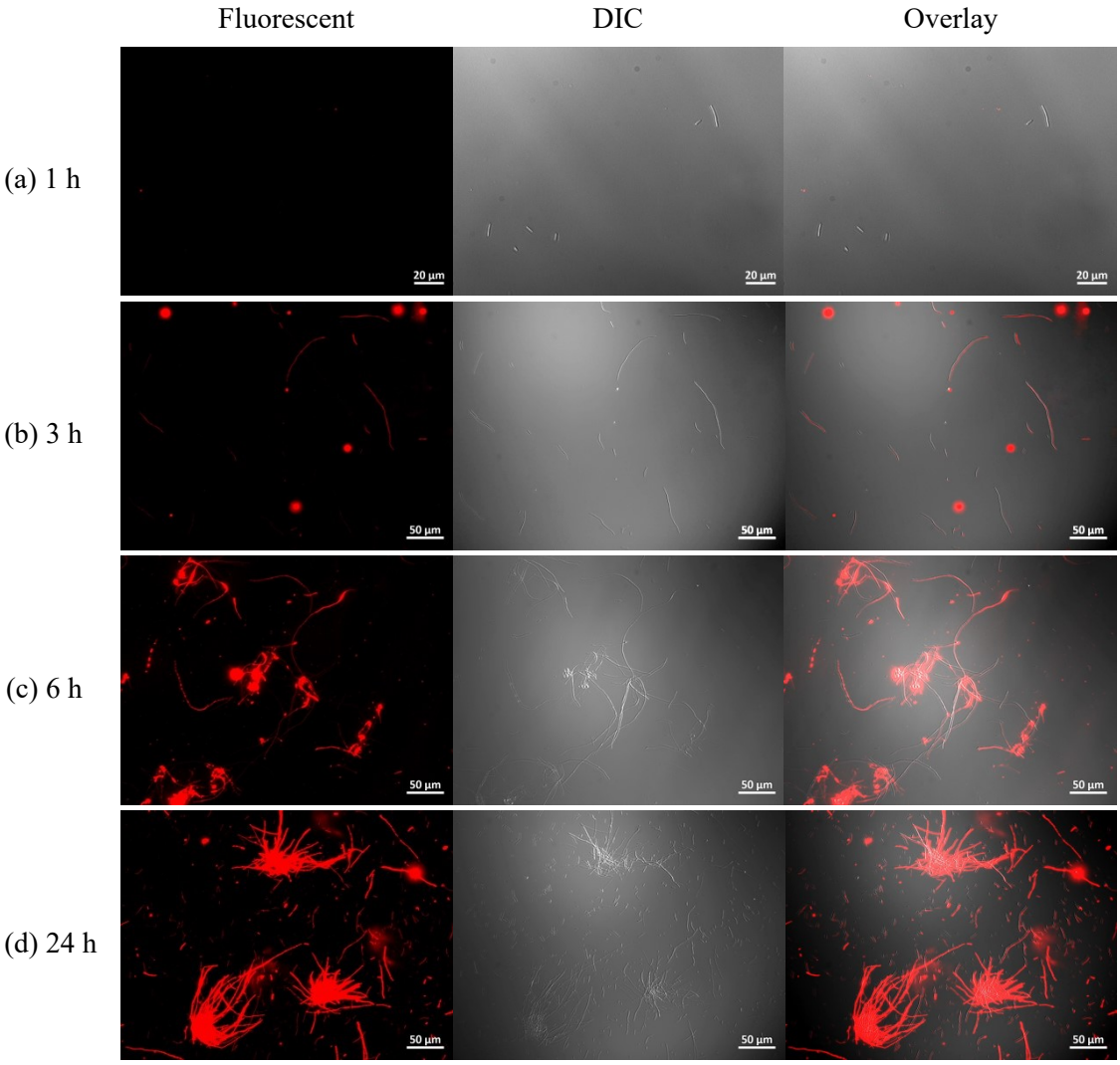


Figure S14. Merged fluorescent and DIC images of *E. coli*/**Et-PTB** after (a) 1 h, (b) 3 h, (c) 6 h, and (d) 24 h incubation.