Supporting Information

One-Step Light-Up Metabolic Probes for *In-Situ* Discrimination and Killing of Intracellular Bacteria

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Table of Contents

Synthetic procedure:

Synthesis of TPEPy

(2-(4-bromophenyl)ethene-1,1,2-triyl)tribenzene (1g, 2.4 mmol), 4-pyridinylboronic acid (360 mg, 0.24 mmol), Pd(PPh3)4 (270mg, 0.24 mmol) were mixed and dissolved in THF (16 mL). Under N_2 atmosphere, aqueous solution of K_2CO_3 (4 mL, 4.5M) was added. The mixture was continuously stirred and heated at 75 °C for 13 h. After cooling to room temperature, the reaction was quenched with water and ethyl acetate and washed with water (20 mL ×3). The organic phase was dried on Na_2SO_4 and solvent was removed under reduced pressure. The obtained residue was purified with chromatography (hexane/ethyl acetate = 1/1, v/v) to give product as creamy white solid (611 mg, 62% yield). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.62 (d, *J* = 6.3 Hz, 2H), 7.50 – 7.44 (m, 2H), 7.42 (d, *J* = 8.4 Hz, 2H), 7.18 – 7.10 (m, 12H), 7.07 (dt, *J* = 6.7, 4.2 Hz, 5H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 150.28 (d, *J* = 7.0 Hz), 147.89, 145.00, 143.59 (d, *J* = 5.3 Hz), 141.93, 140.19, 135.78, 132.24, 131.69 – 131.23 (m),

128.17 – 127.72 (m), 127.03 – 126.55 (m), 126.29, 121.42, 77.47, 77.15, 76.83. High resolution ESI-MS, m/z: $[M-Br]^+$ calculated 409.1825, found 410.1908.

Synthesis of TPEPy-butyne

TPEPy (100 mg, 0.24 mmol) was dissolved in DMF (5 mL). Under N_2 atmosphere, 4-bromo-1-butyne (115 μL, 1.2 mmol) was injected and the mixture was stirred and heated at 80 °C for 15 h. After cooling to room temperature, the mixture was separated with chromatography directly (Eluent: hexane/ethyl acetate = 1/5, v/v, dichloromethane/methanol = 100/1, v/v) to get product as yellow solid (77 mg, 59% yield). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.40 (d, *J* = 6.4 Hz, 2H), 8.09 (d, *J* = 6.3 Hz, 2H), 7.55 (d, *J* = 8.4 Hz, 2H), 7.16 – 7.00 (m, 17H), 5.12 (t, *J* = 6.0 Hz, 2H), 3.08 (q, *J* = 5.2 Hz, 2H), 2.11 (t, *J* = 2.5 Hz, 1H). High resolution ESI-MS, m/z: $[M-Br]^+$ calculated 462.2216, found 462.2243.

Synthesis of TPEPy-Ala

TPEPy-butyne (20 mg, 0.037 mmol) and 3-azido-D-alanine hydrochloride (6.2 mg, 0.037 mmol) were dissolved in nitrogen degassing DMSO (0.5 mL). Copper(I) bromide (5.3 mg, 0.037mmol) was dispersed in 0.1 mL trimethylamine and injected into the solution. The reaction was stirred at room temperature for 10 h. The mixture was filtrated by 0.45 μm nylon membrane filter and subjected to HPLC for purification. TPEPy-Ala was obtained as yellow powder (7.2 mg, 30% yield). ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.67 (d, *J* = 6.4 Hz, 2H), 8.30 (d, *J* = 6.3 Hz, 2H), 7.82 (m, *J* = 8.6 Hz, 3H), 7.29 (d, *J* = 8.1 Hz, 2H), 7.15 (t, *J* = 5.5, 2.5 Hz, 8H), 7.09 – 7.03 (m, 6H), 4.95 (s, 2H), 4.18 (s, 1H), 3.45 (s, 2H), 2.68 (s, 1H). MALDI-TOF-MS, m/z: $[M-Br]^+$ calculated 592.2707, found 592.2840.

Synthesis of TPAPy

4-bromo-*N*,*N*-diphenylaniline (1 g, 3.08 mmol), 4-pyridinylboronic acid (391 mg, 3.18 mmol), Pd(PPh3)4 (355 mg, 3.08 mmol) were mixed and dissolved in THF (16 mL). Under N_2 atmosphere, aqueous solution of K_2CO_3 (4 mL, 4.5M) was added. The mixture was continuously stirred and heated at 75 °C for 13 h. After cooling to room temperature, the reaction was quenched with water and ethyl acetate and washed with water (20 mL ×3). The organic phase was dried on Na_2SO_4 and solvent was removed under reduced pressure. The obtained residue was purified with chromatography (hexane/ethyl acetate = 1/1, v/v) to yield pale-yellow solid (630 mg, 63% yield). ^{1}H

NMR (400 MHz, Chloroform-*d*) δ 8.69 – 8.58 (m, 2H), 7.55 – 7.47 (m, 4H), 7.30 (d, *J* = 15.8 Hz, 4H), 7.17 – 7.11 (m, 6H), 7.11 – 7.06 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 150.29, 149.04, 147.73, 147.34, 131.02, 129.54, 127.74, 125.08, 123.71, 123.01, 120.97, 77.48, 77.16, 76.84. High resolution ESI-MS, m/z: $[M\text{-}Br]^+$ calculated 322.1464, found 323.1545.

Synthesis of TPAPy-butyne

TPAPy (100 mg, 0.31 mmol) was dissolved in DMF (5 mL). Under N_2 atmosphere, 4-bromo-1-butyne (145 μL, 1.55 mmol) was injected and the mixture was stirred and heated at 80 °C for 8 h. After cooling to room temperature, the mixture was separated with chromatography directly (Eluent: hexane/ethyl acetate = 1/5, v/v, dichloromethane/methanol = 100/1, v/v) to get the product as orange solid (63 mg, 46% yield). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.24 (s, 2H), 7.67 (s, 2H), 7.37 (s, 4H), 7.20 (s, 8H), 5.02 (s, 2H), 3.07 (s, 2H), 2.12 (s, 1H). High resolution ESI-MS, m/z: $[M\text{-}Br]^+$ calculated 375.1855, found 375.1858.

Synthesis of TPAPy-Kdo

TPAPy-butyne (20 mg, 0.044 mmol) and 8-azido-3,8-dideoxy-D-manno-octulosonic acid (12.3 mg, 0.044 mmol) were dissolved in nitrogen degassing DMSO (0.5 mL). Copper(I) bromide (6.3 mg, 0.044 mmol) was dispersed in 0.15 mL trimethylamine and injected into the solution. The reaction was stirred at room temperature for 10 h. The mixture was filtrated by 0.45 μm nylon membrane filter and subjected to HPLC for purification. TPAPy-Kdo was obtained as orange powder (8 mg, 26% yield). ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.59 (d, *J* = 15.0 Hz, 2H), 8.20 (d, *J* = 6.7 Hz, 2H), 7.88 (d, *J* = 9.3 Hz, 3H), 7.39 (t, *J* = 7.7 Hz, 5H), 7.23 – 7.18 (m, 7H), 7.06 (d, *J* = 8.5 Hz, 2H), 5.22 (s, 2H), 3.66 (s, 2H), 3.48 (s, 1H), 3.21 (q, *J* = 7.3 Hz, 5H), 2.69 (s, 1H), 2.04 (d, *J* = 12.1 Hz, 2H), 1.68 (s, 1H). MALDI-TOF-MS, m/z: $[M\text{-}Br]^+$ calculated 638.2609, found 638.2418.

ROS measurement

For ROS measurement, 750 μL of 40 μM DCFH was mixed into 10 μM of TPEPy-Ala, TPEPy-Kdo or Ce6 in PBS solution and irradiated by white light. After the different irradiation time, the emission signal was measured one by one with an excitation of 480 nm. For singlet oxygen detection, 50 μM of ABDA was mixed with 10 μM TPEPy-Ala, TPEPy-Kdo, or Rose bengal in PBS solution and irradiated by

white light. The absorbance of ABDA mixed with TPEPy-Ala, TPEPy-Kdo or Rose bengal solution was recorded one by one every 60 s.

SEM studies

S. aureus and *E. coli* bacterial cells were incubated with 10 μM TPEPy-Ala and TPAPy-Kdo respectively for 30 min and then illuminated in white light for 30 min. The bacteria solution was then treated with 5% glutaraldehyde at 4 °C overnight, followed by ethanol gradient dehydration for SEM studies. Bacteria without treatment were also imaged for comparison.

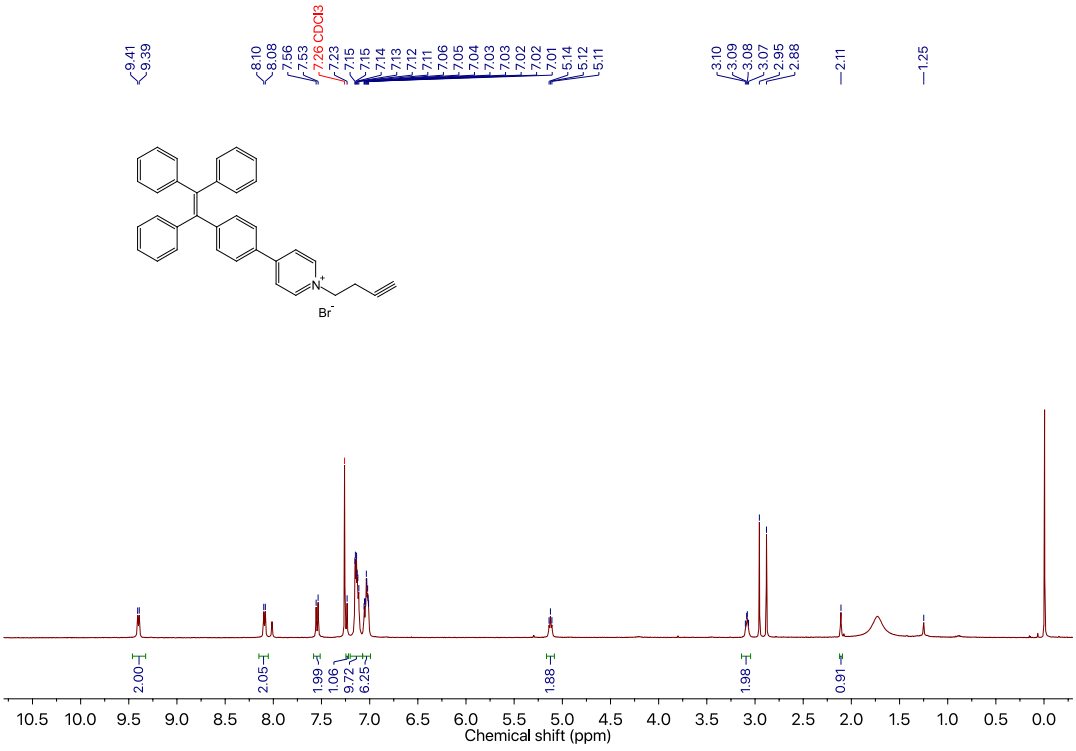

Fig. S1. ^{1}H NMR spectrum of TPEPy-butyne.

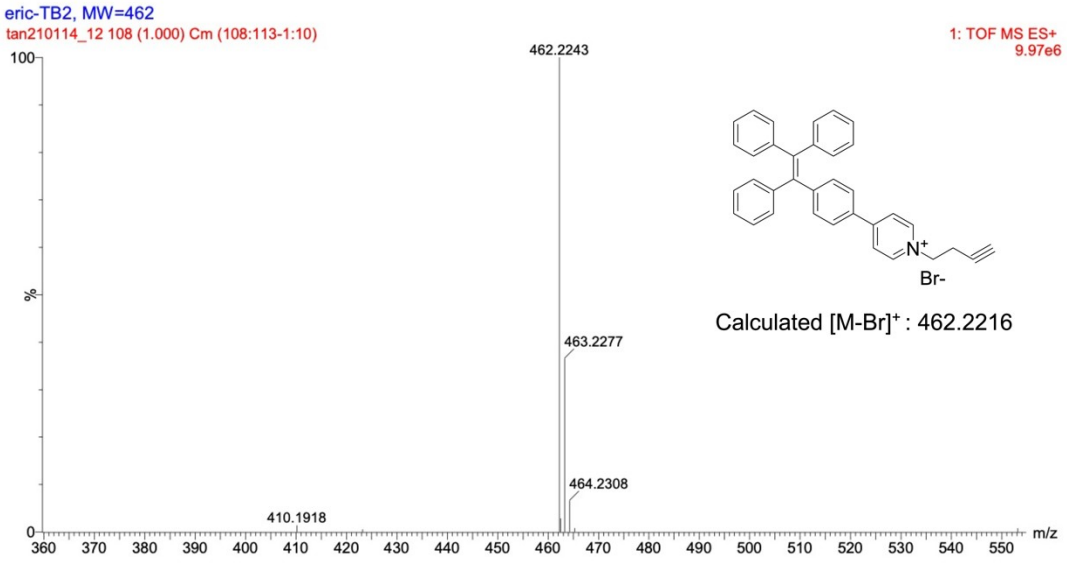

Fig. S2. ESI-mass spectrum of TPEPy-butyne.

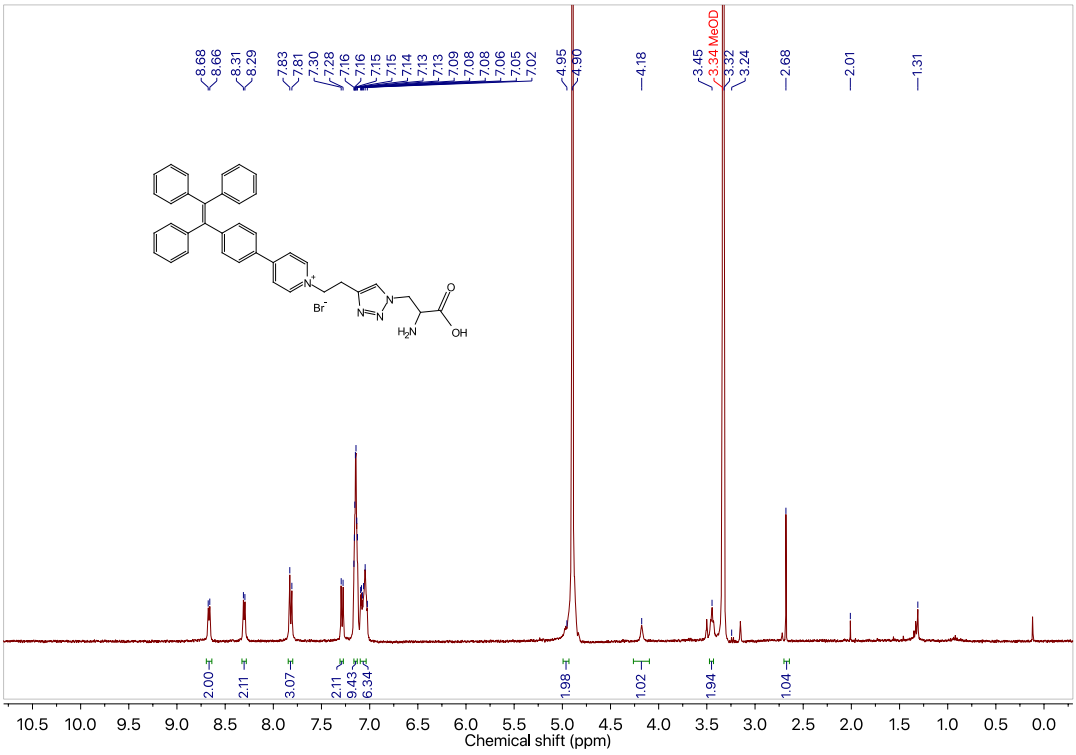

Fig. S3. ^{1}H NMR spectrum of TPEPy-Ala.

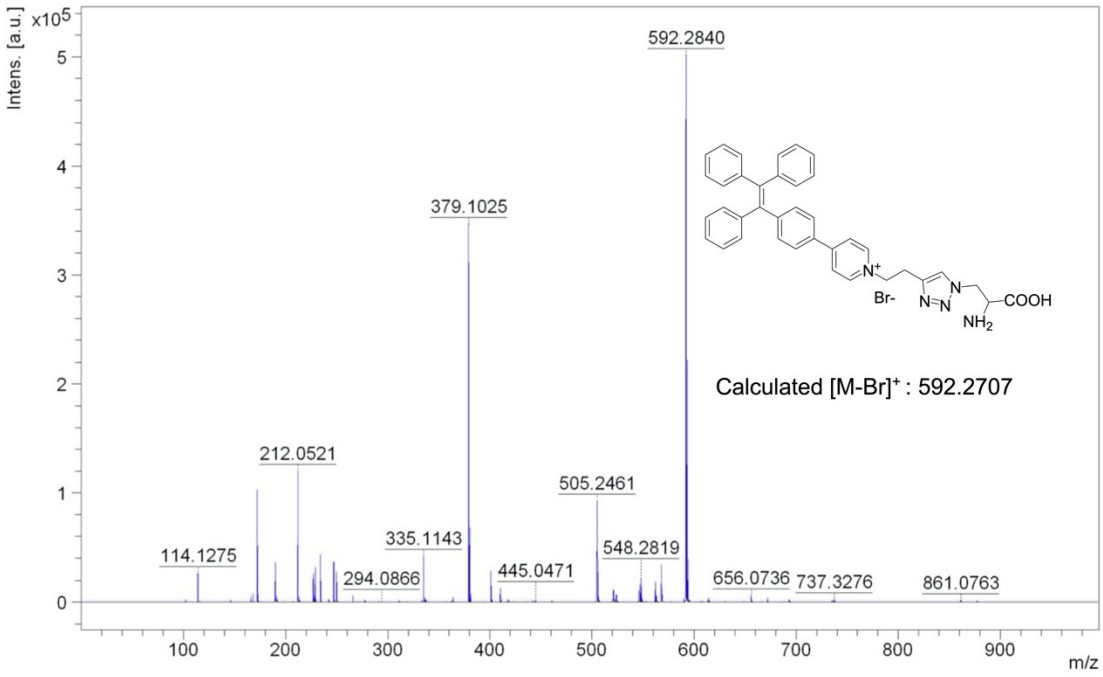

Fig. S4. MALDI-TOF mass spectrum of TPEPy-Ala.

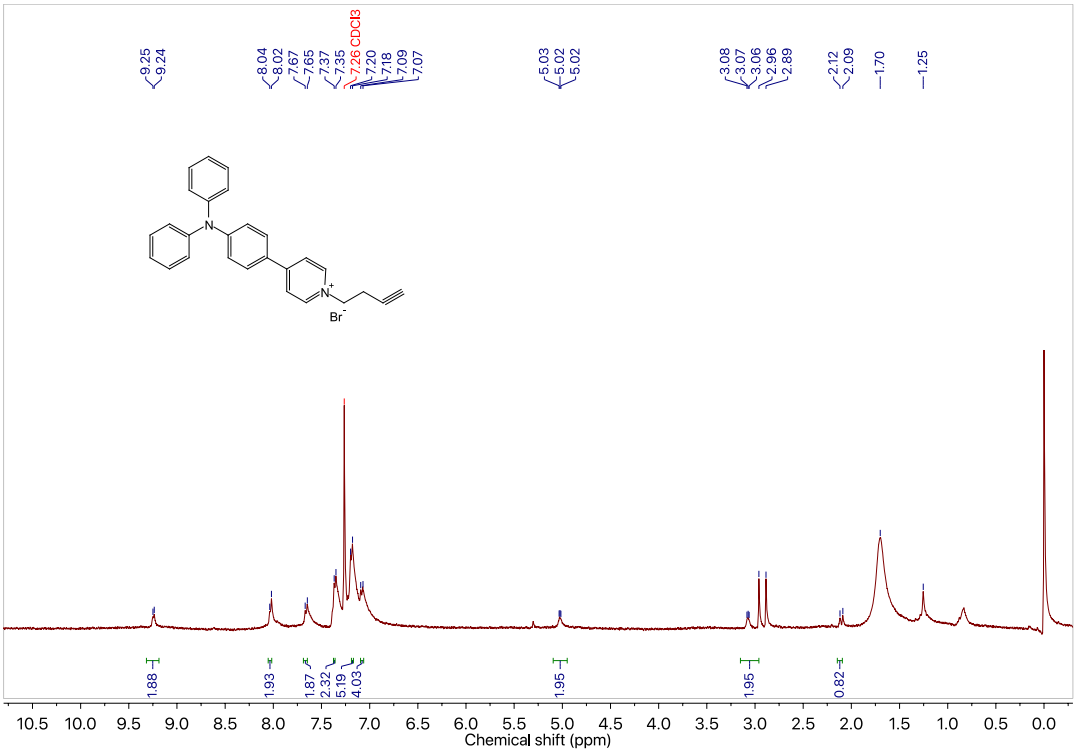

Fig. S5. ^{1}H NMR spectrum of TPAPy-butyne.

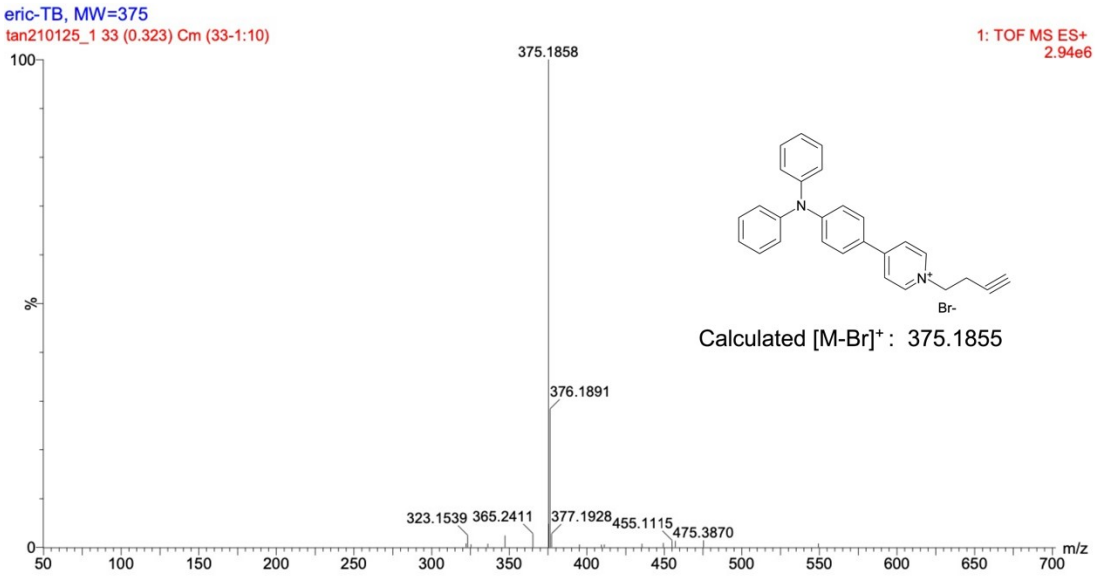

Fig. S6. ESI-mass spectrum of TPAPy-butyne.

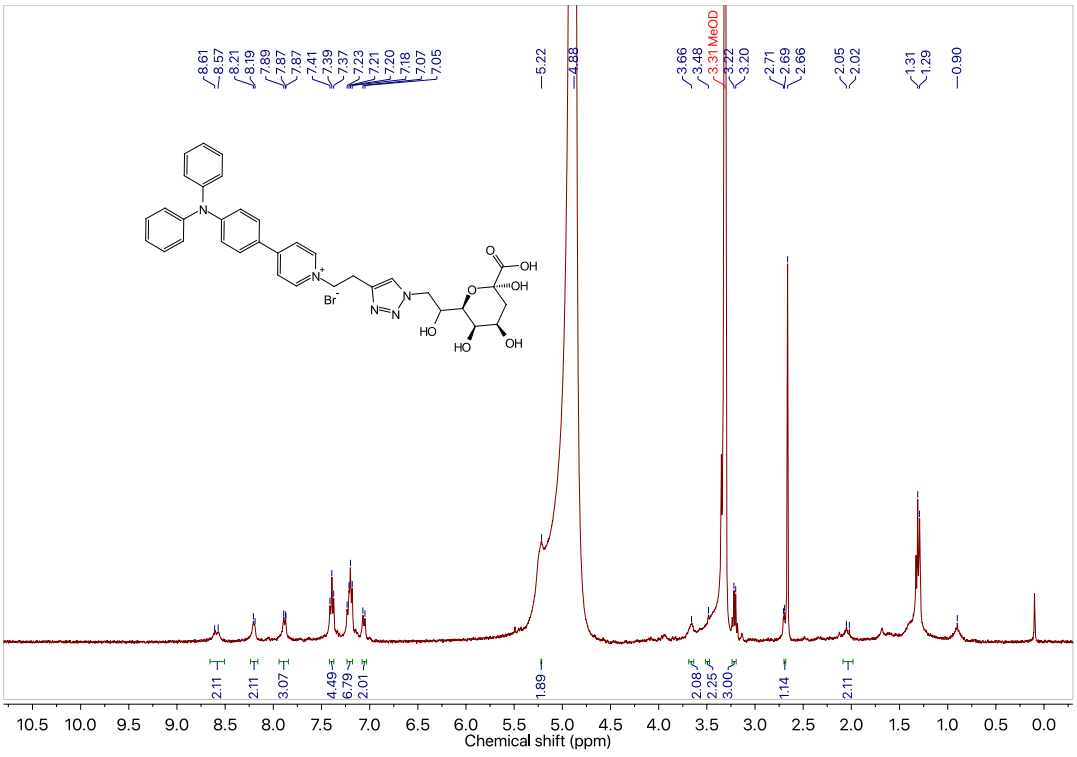

Fig. S7. ^{1}H NMR spectrum of TPAPy-Kdo.

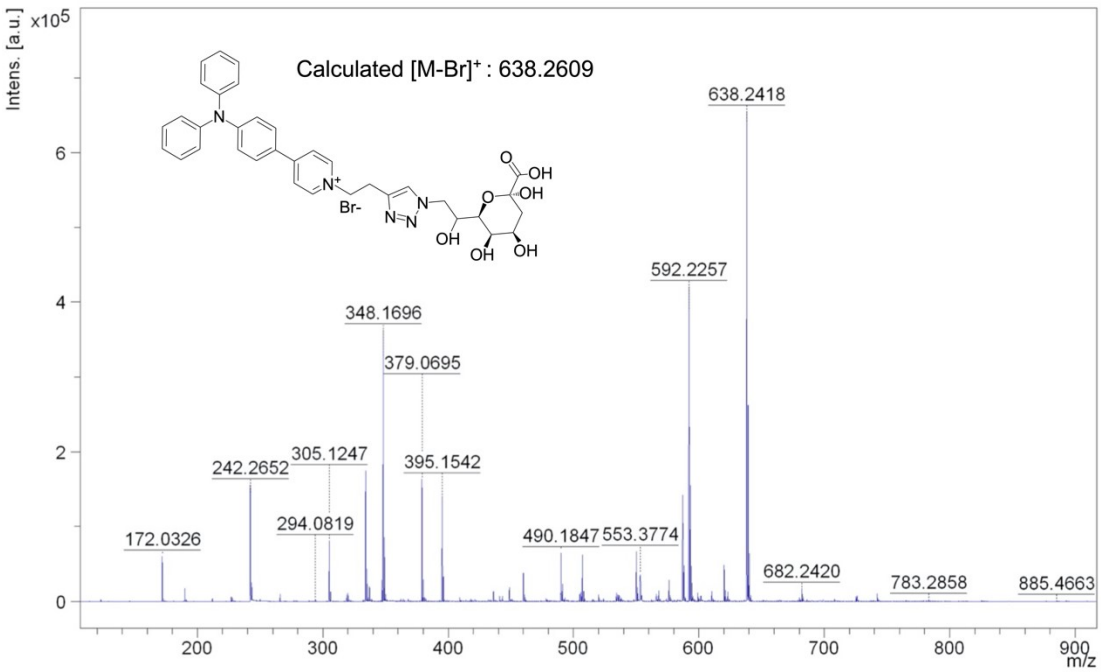

Fig. S8. MALDI-TOF mass spectrum of TPAPy-Kdo.

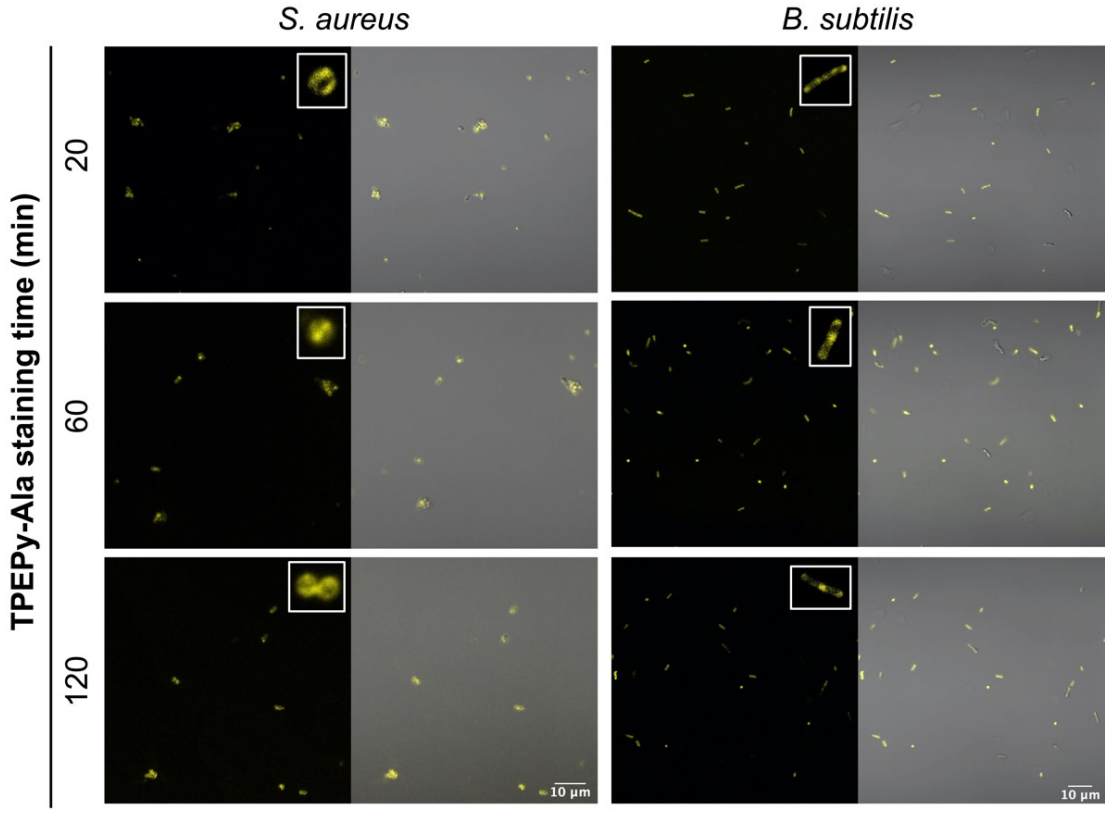

Fig. S9. CLSM and merged images of *S. aureus* and *B. subtilis* incubated with TPEPy-Ala for 20 min, 60 min and 120 min (Concentration: 10 μM, λ_{ex} = 405 nm, 2.0% laser power, λ_{em} = 500-650 nm) Scale: 10 μm.

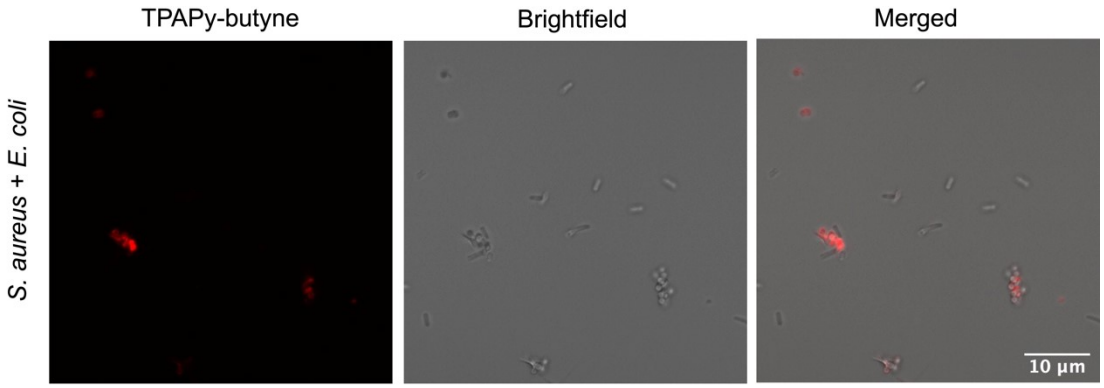

Fig. S10. CLSM, brightfield and merged images of mixed *S. aureus* and *E. coli* incubated with TPAPy-butyne for 2 h (Concentration: 10 µM λ_{ex} = 488 nm, 2.0% laser power, λ_{em} : 550−700 nm) Scale: 10 µm.

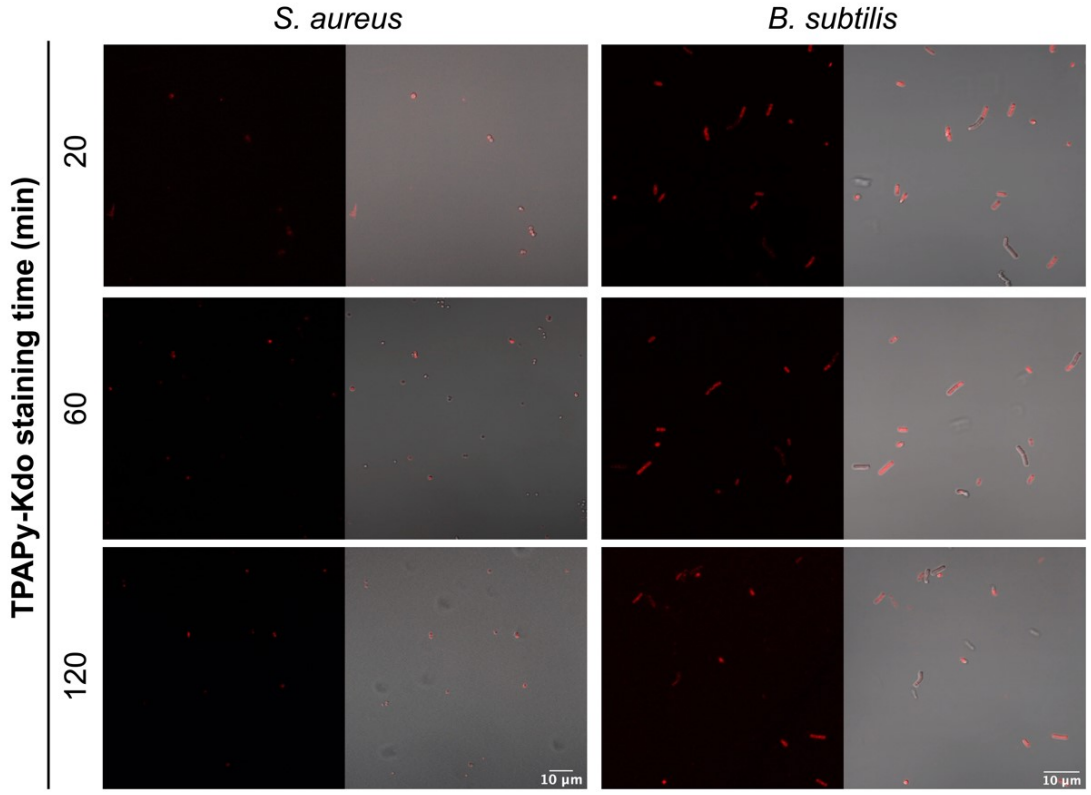

Fig. S11. CLSM and merged images of *S. aureus* and *B. subtilis* incubated with TPAPy-Kdo for 20 min, 60 min and 120 min (Concentration: 10 µM, λ_{ex} = 488 nm, 2.0% laser power, λ_{em} = 550-700 nm) Scale: 10 µm.

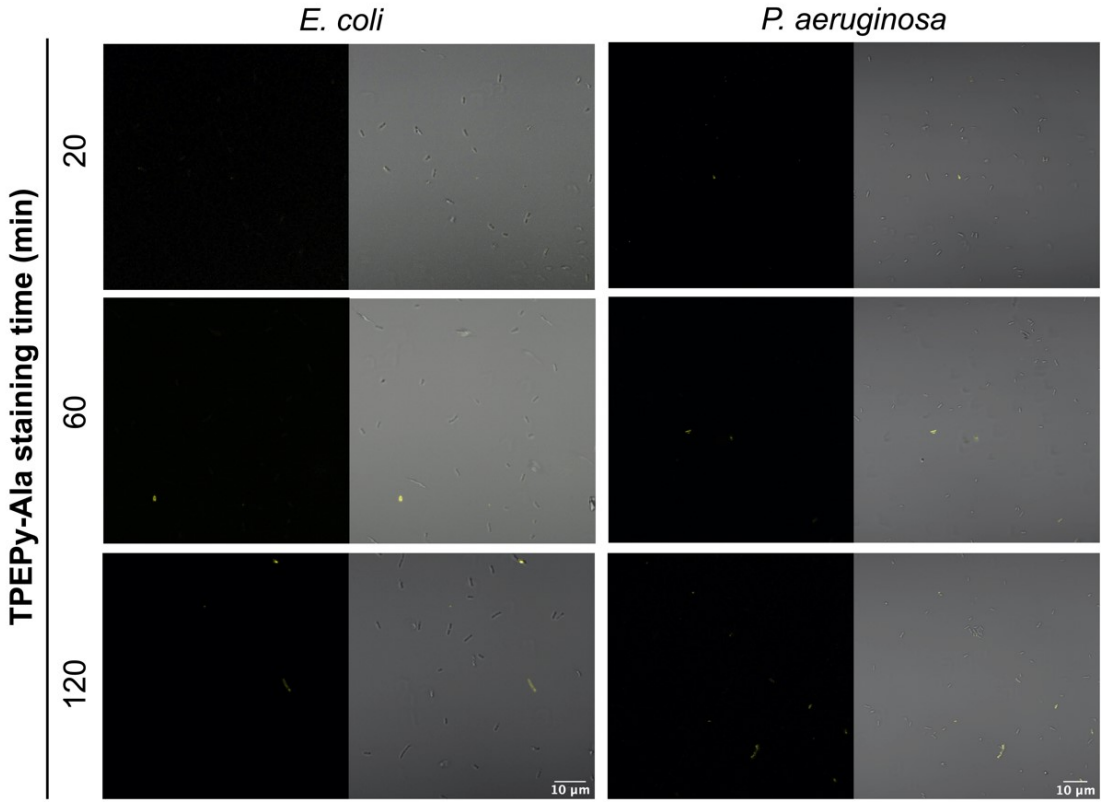

Fig. S12. CLSM and merged images of *E. coli* and *P. aeruginosa* incubated with TPEPy-Ala for 20 min, 60 min and 120 min (Concentration: 10 μM, λ_{ex} = 405 nm, 2.0% laser power, λ_{em} = 500-650 nm) Scale: 10 μm.

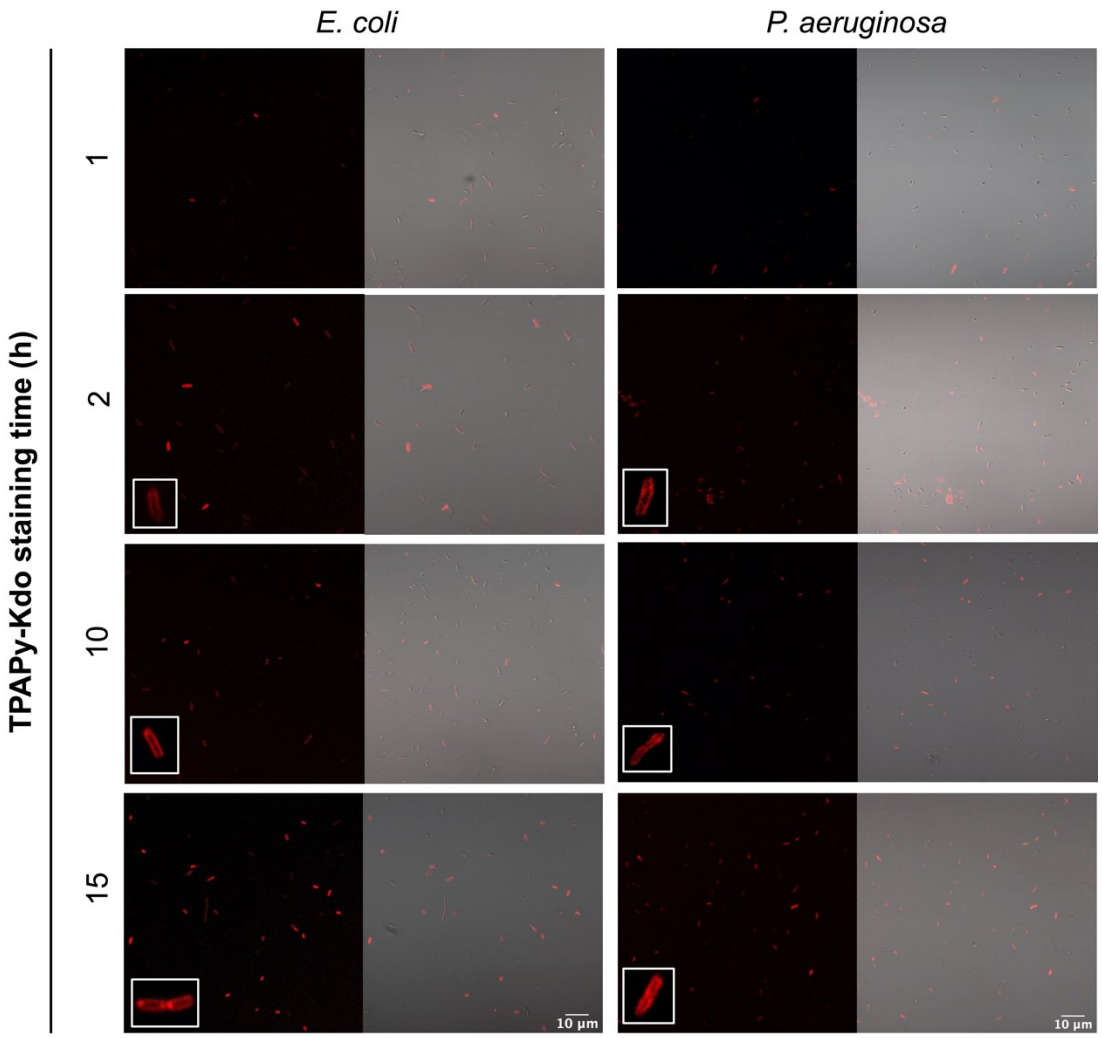

Fig. S13. CLSM and merged images of *E. coli* and *P. aeruginosa* incubated with TPAPy-Kdo for 1 h, 2 h, 10 h and 15 h (Concentration: 10 μM, λ_{ex} = 488 nm, 2.0% laser power, λ_{em} = 550-700 nm) Scale: 10 μm.

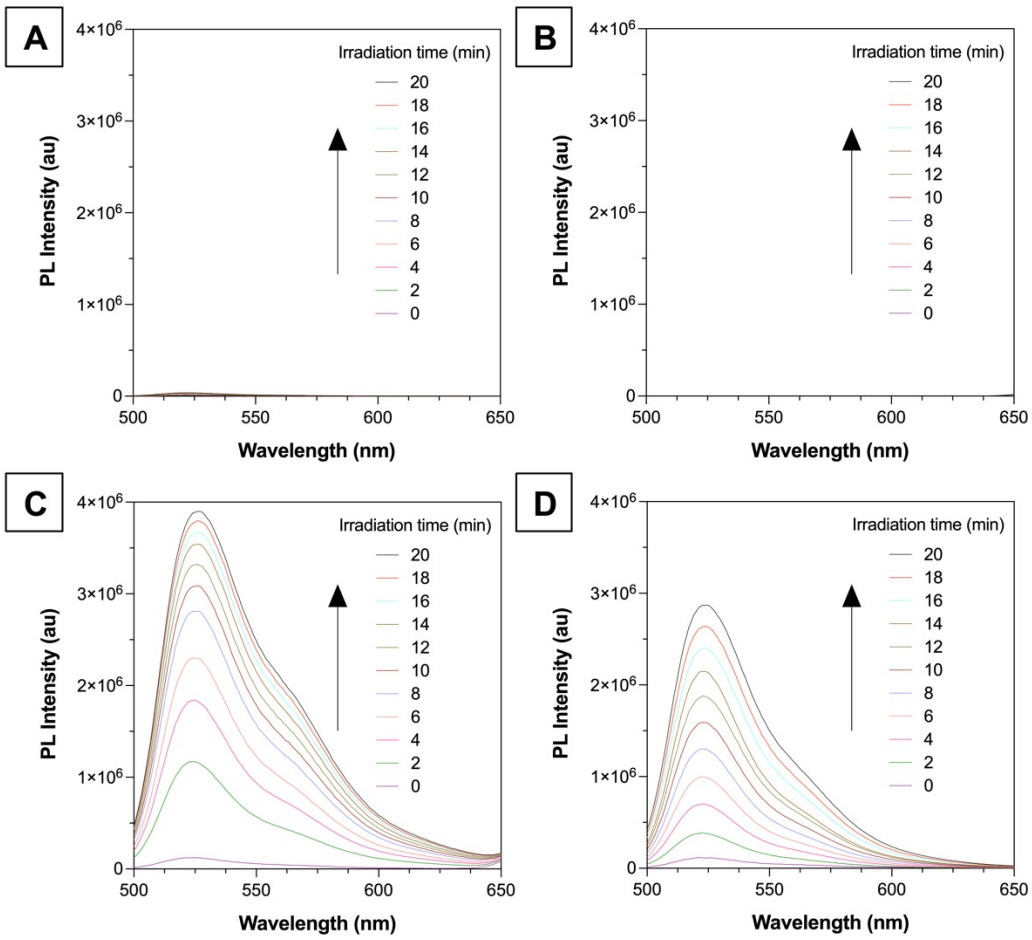

Fig. S14. PL spectra of (A) DCFH (B) Ce6 (C) mixture of DCFH and Ce6 and (D) mixture of DCFH and TPAPy-Kdo in PBS upon white light irradiation (10 mW/cm^2) for various time interval.

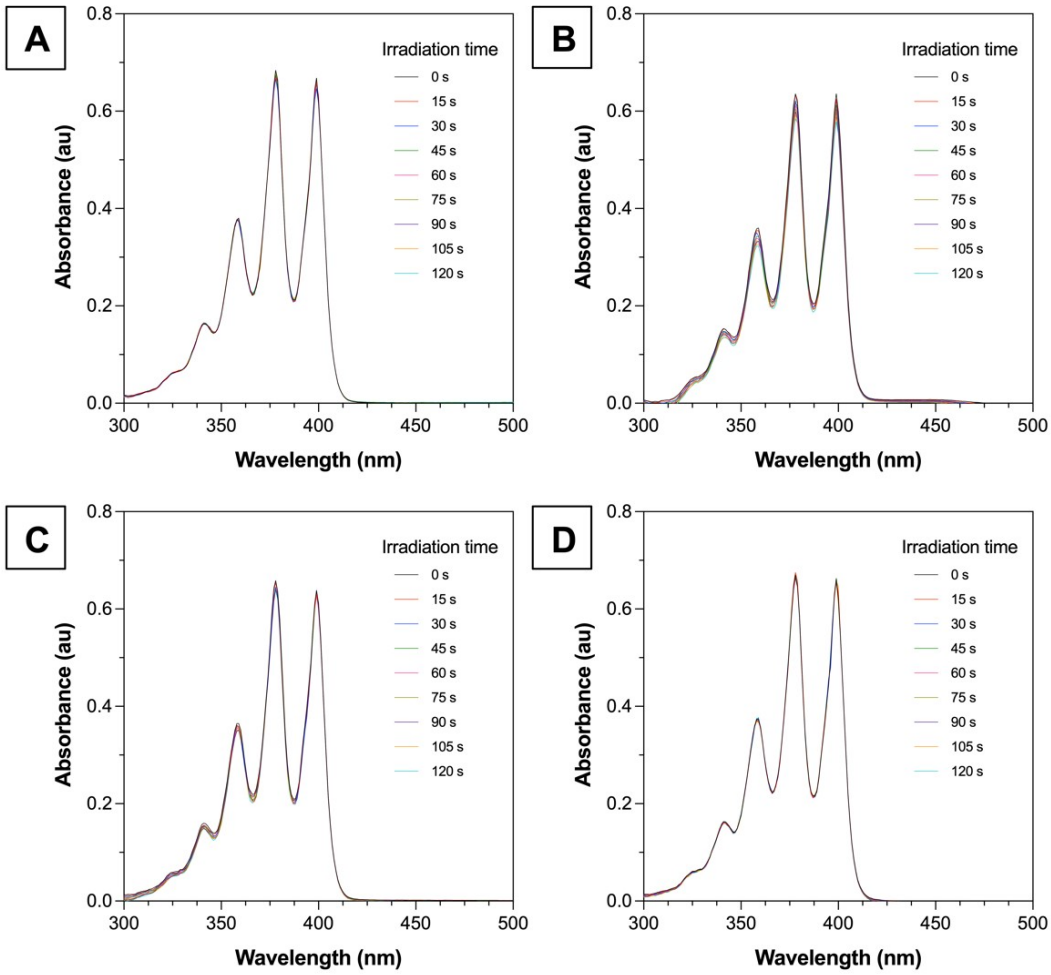

Fig. S15. UV-Vis spectra of (A) ABDA (B) mixture of Rose Bengal and ABDA (C) mixture of ABDA and TPEPy-Ala and (D) mixture of ABDA and TPAPy-Kdo in 1/99 DMSO-water upon white light irradiation (10 mW/cm^2) for various time interval.

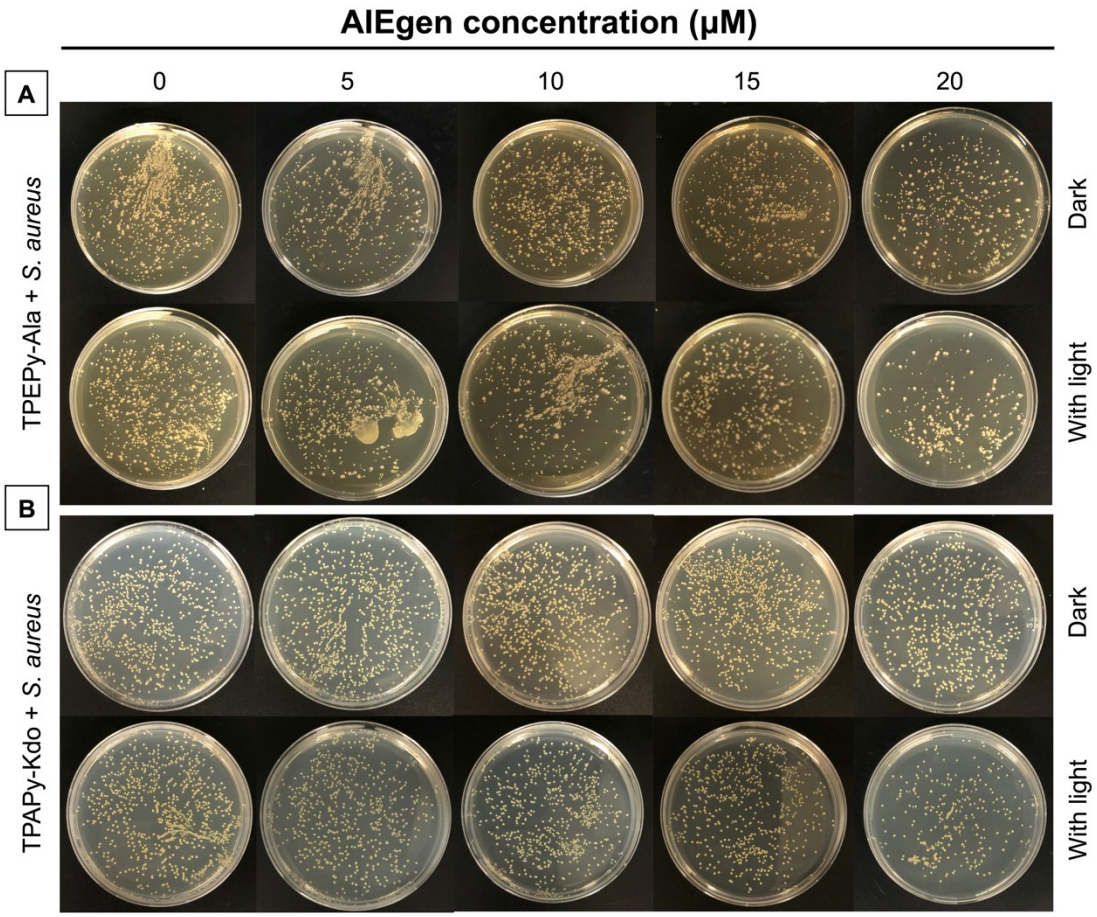

Fig. S16. *S. aureus* colonies on LB broth agar plates treated with (A) TPEPy-Ala and (B) TPAPy-Kdo for 30 min with and without white light irradiation for 30 min.

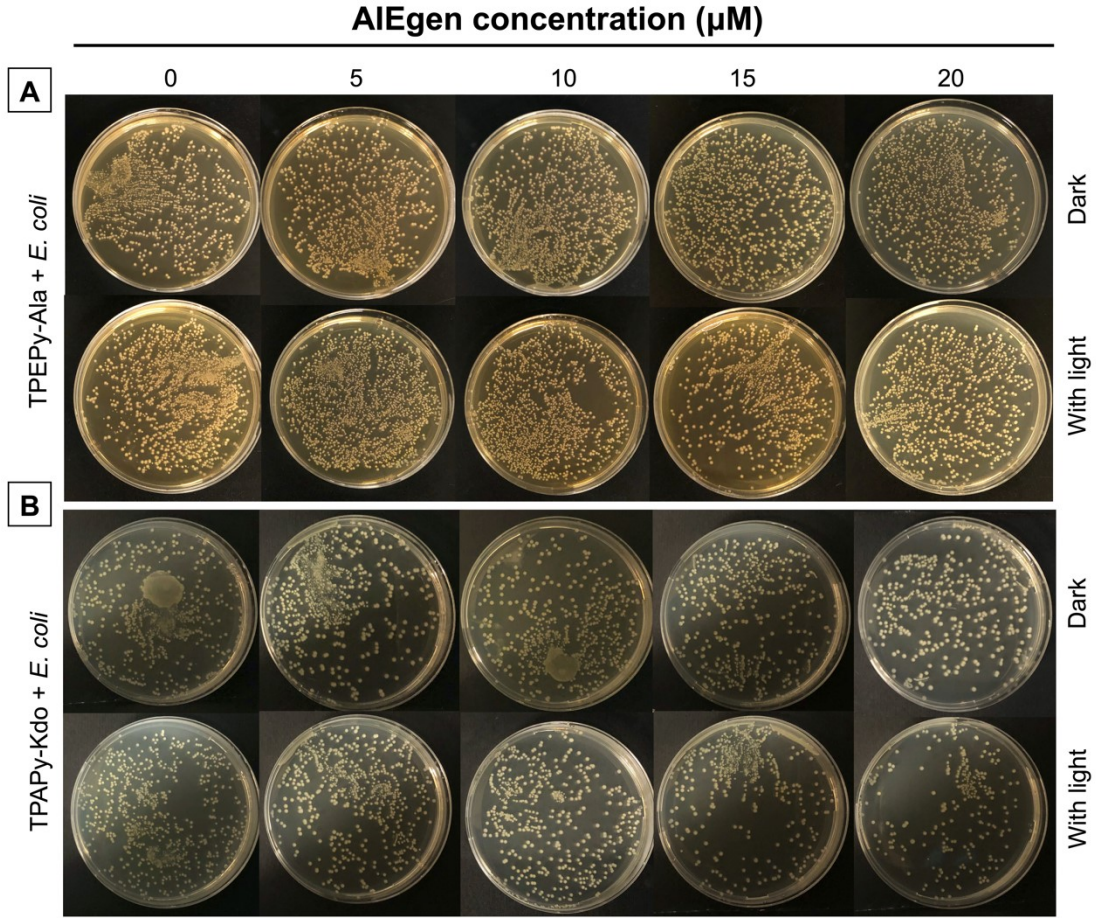

Fig. S17. *E. coli* colonies on LB broth agar plates treated with (A) TPEPy-Ala and (B) TPAPy-Kdo for 30 min with and without white light irradiation for 30 min.

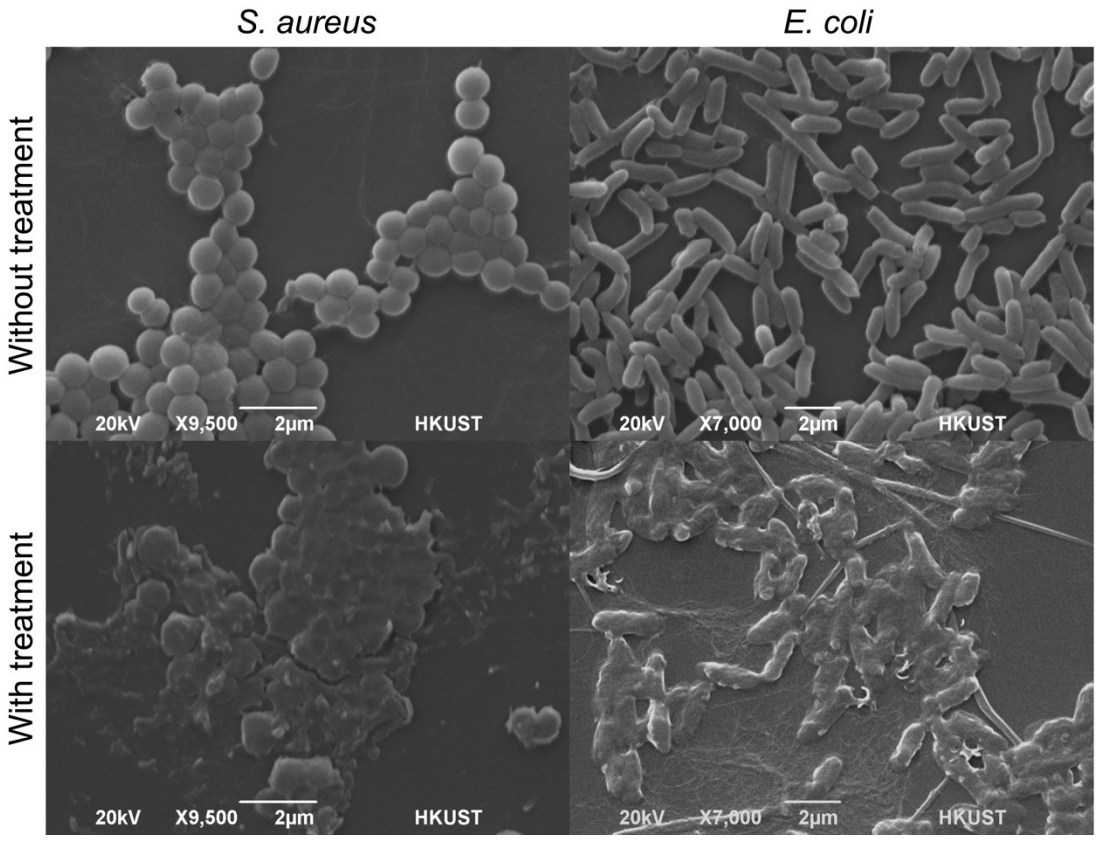

Fig. S18. SEM images of *S. aureus* and *E. coli* without treatment and with treatment by TPEPy-Ala and TPAPy-Kdo respectively for 30 min, followed by 30 min of white light irradiation (10 mW cm^2). Scale bar: 2 μm.

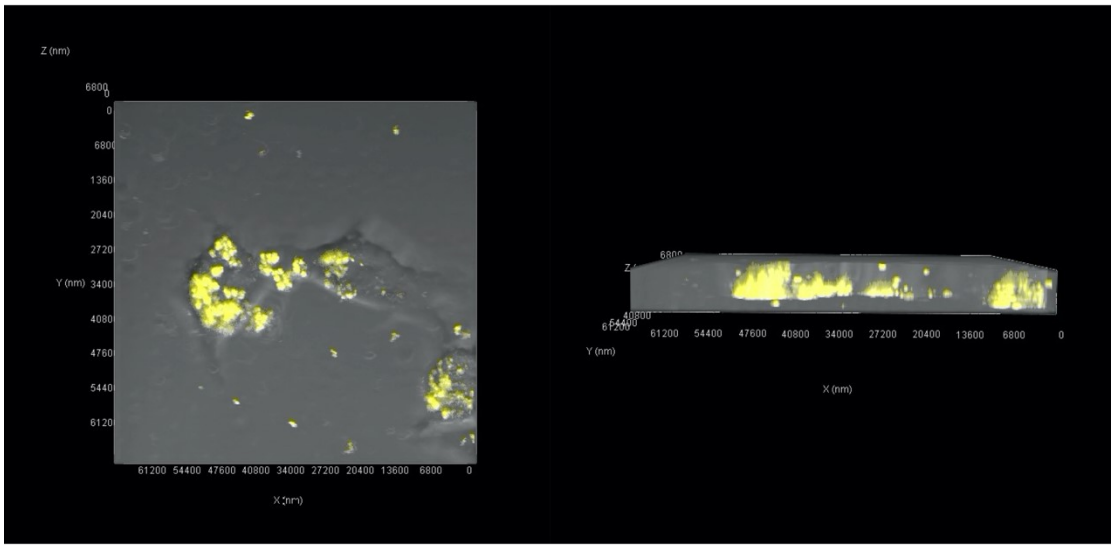

Fig. S19. 3D CLSM images of *S. aureus* infected Raw264.7 cells incubated with TPEPy-Ala for 1 h (Concentration: 10 μM, λ_{ex} = 405 nm, 2.0% laser power, λ_{em} : 500−650 nm).

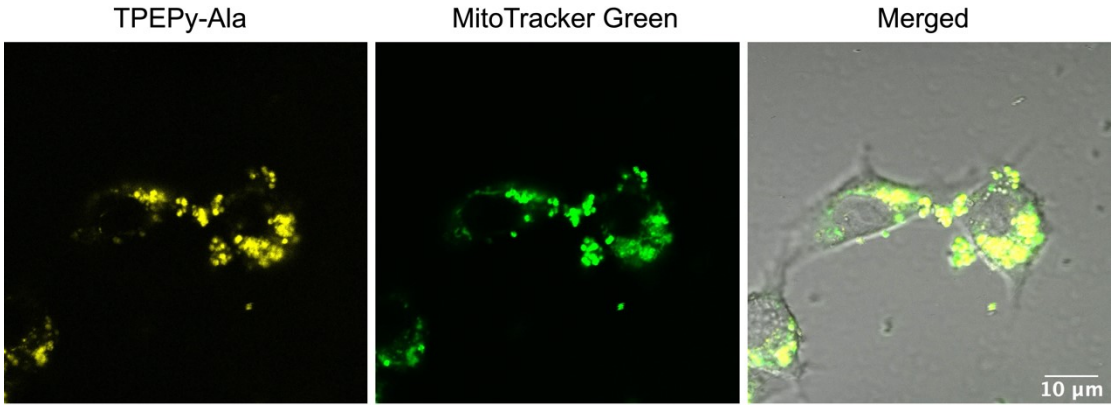

Fig. S20. Co-localization imaging of Raw264.7 cell with intracellular *S. aureus* stained with TPEPy-Ala for 1 h (Concentration: 10 μM, λ_{ex} = 405 nm, 2% laser power, λ_{em} = 500-650 nm) and MitoTracker Green for 15 min (Concentration: 500 nM, λ_{ex} = 488 nm, 2% laser power, λ_{em} = 500-530 nm) Scale: 10 μm.

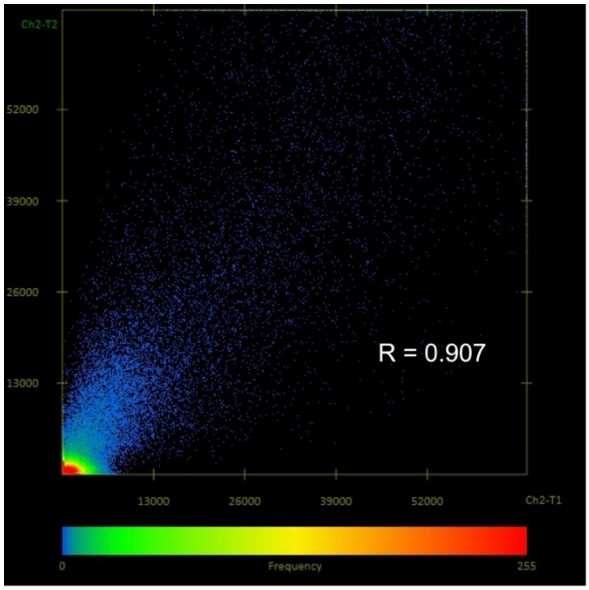

Fig. S21. Scatter plot indicating a correction coefficient between TPEPy-Ala and MitoTracker Green of Fig. S20 Pearson correlation coefficient R = 0.907.

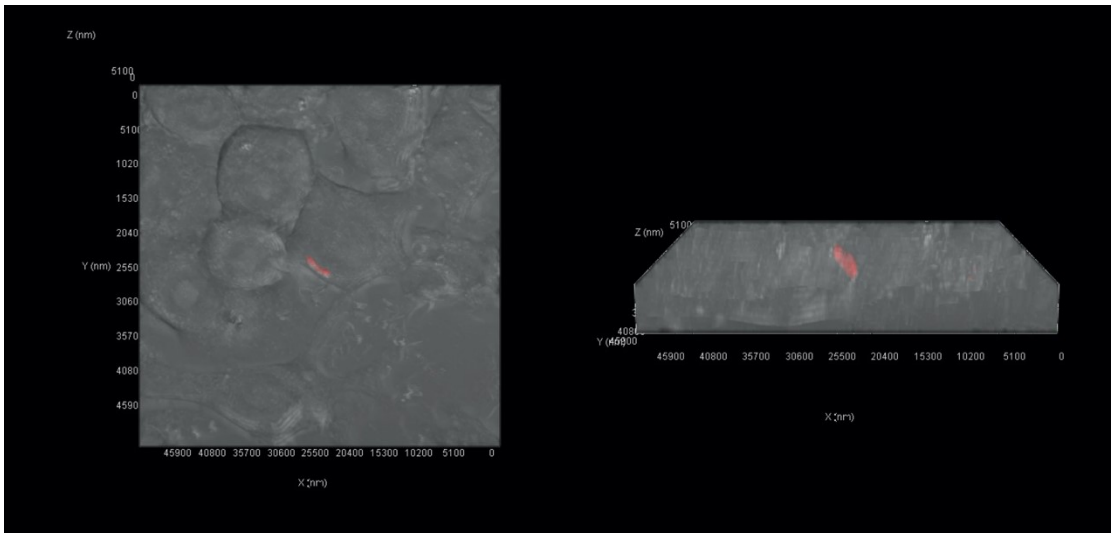

Fig. S22. 3D CLSM images of *E. coli* infected Raw264.7 cells incubated with TPAPy-Kdo for 2 h (Concentration: 10 μM, λ_{ex} = 488 nm, 2.0% laser power, λ_{em} : 550−700 nm).

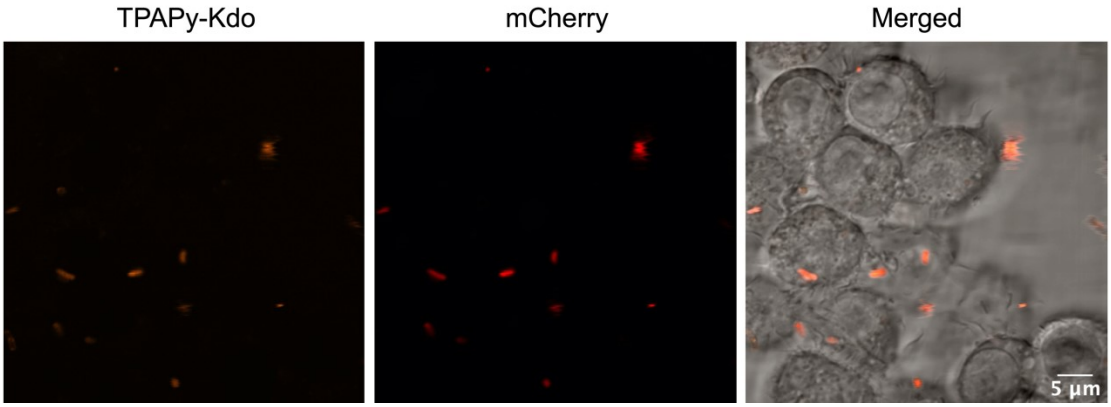

Fig. S23. Co-localization imaging of Raw264.7 cell with intracellular mCherry-expressing *E. coli* (λ_{ex} = 561 nm, 1.5% laser power, λ_{em} = 600-700 nm) stained with TPAPy-Kdo for 2 h (Concentration: 10 µM, λ_{ex} = 488 nm, 2.0 % laser power, λ_{em} = 550-700 nm) Scale: 5 µm.

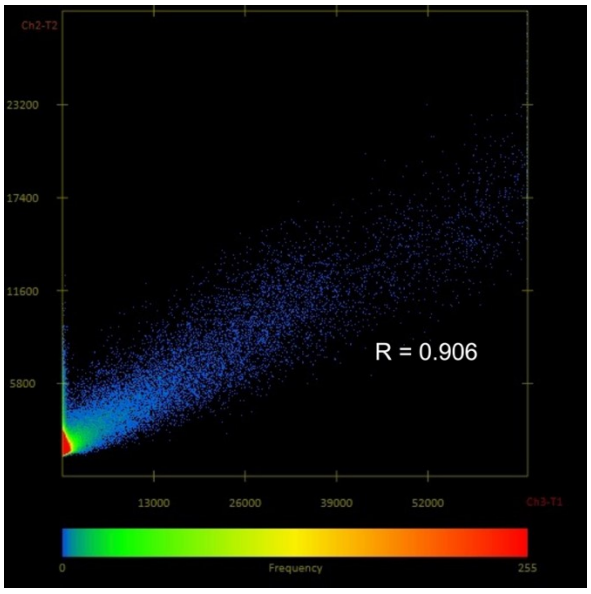

Fig. S24. Scatter plot indicating a correction coefficient between TPAPy-Kdo and mCherry-expressing *E. coli* of Fig. S23. Pearson correlation coefficient R = 0.906.

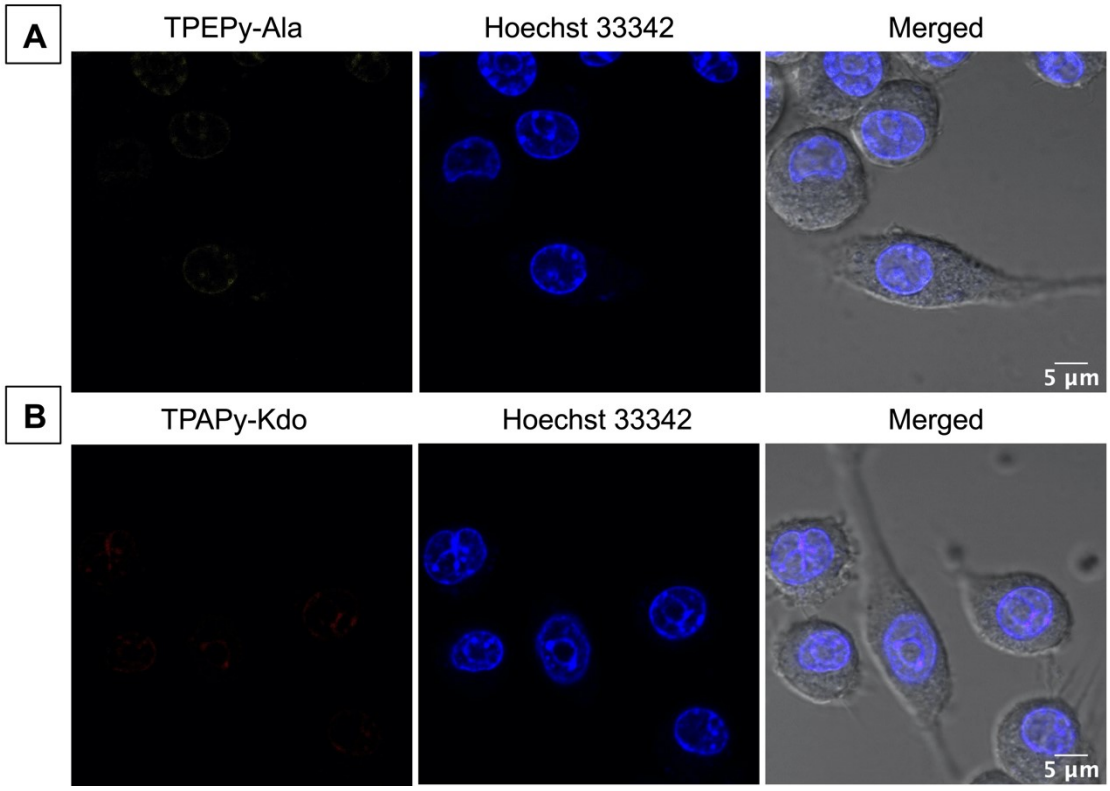

Fig. S25. CLSM and merged images of non-infected Raw264.7 cells incubated with (A) TPEPy-Ala for 2 h (Concentration: 10 µM, λ_{ex} = 405 nm, 2% laser power, λ_{em} = 500-600 nm) (B) TPAPy-Kdo for 2 h (Concentration: 10 µM, λ_{ex} = 488 nm, 2.0 % laser power, λ_{em} = 550-700 nm) and co-stained with Hoechst 33342 for 15 min (Concentration: 1 µM, λ_{ex} = 405 nm, 2% laser power, λ_{em} = 430–500 nm) Scale: 5 µm.