Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information for

Real Time Detection of Live Microbes with a Highly Sensitive Bioluminescent Nitroreductase Probe

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1. Synthesis

General. All solvents were of reagent grade. THF was dried by passing through activated alumina. All commercially purchased chemicals were used as received. ^{1}H and ^{13}C NMR spectra were obtained from a Bruker AVB-400 NMR spectrometer. Signals were internally referenced to solvent residues. Low resolution mass spectral analyses were carried out using a LCMS (Agilent Technology 6130, Quadrupole LC/MS).

Synthesis of **1a**. A solution of 6-amino-2-cyano-benzothiazole (30 mg, 0.17 mmol) and DMAP (25 mg, 0.20 mmol) in THF (40 ml) was heated to 70 °C. A solution of 4-nitrobenzyl chloroformate (48 mg, 22 mmol) in (10 ml) was slowly added. The mixture was heated at 70 °C for overnight. The mixture was cooled to room temperature, filtered and concentrated. The residue was purified by a silica column (EtOAc:hexanes = 1:1). The product was obtained as a white solid. Yield = 30 mg, 50%. ^{1}H NMR (400 MHz, d_6-acetone, 298 K): ŭ (ppm) 9.51 (s, 1 H), 8.61 (s, 1H), 8.28 (d, *J* = 8.8 Hz, 2 H), 8.17 (d, *J* = 9.2 Hz, 1 H), 7.79 (d, *J* = 9.2 Hz, 1 H), 7.75 (d, *J* = 8.8 Hz, 2 H), 5.41 (s, 2 H). $^{13}C\{^{1}H\}$ NMR (100.6 MHz, d_6-acetone, 298 K)): ŭ (ppm) 129.4, 126.0, 124.5, 120.9, 110.7, 66.1.

Synthesis of **1**. To a Schlenk flask containing a solution of **1a** (50 mg, 0.14 mmol) in EtOAc (10 ml) was added a degassed solution of D-cysteine hydrochloride (27 mg, 0.15 mmol) and K_2CO_3 (23 mg, 0.17 mmol) in water (1 ml) under N_2. Degassed MeOH was added to the mixture until a homogeneous solution was formed. The mixture was stirred for 2 hours. The solution was concentrated on a rotary evaporator. 1 M HCl was added dropwisely to the concentrated solution. The yellow precipitate formed was collected by filtration, washed with water and cold MeOH and dried under N_2. Yield = 41 mg, 65%. ^{1}H NMR (400 MHz, d_6-DMSO, 298 K): ŭ (ppm) 10.34 (s, 1 H), 8.34 (s, 1H), 8.28 (d, *J* = 8.4 Hz, 2 H), 8.05 (d, *J* = 9.2 Hz, 1 H), 7.72 (d, *J* = 8.4 Hz, 2 H), 7.58 (d, *J* = 9.2 Hz, 1 H), 5.35 (s, 2 H), 5.10ؚ5.06 (m, 1 H), 3.74ؚ3.70 (m, 1 H), 3.60ؚ3.55 (m, 1 H). $^{13}C\{^{1}H\}$ NMR (100.6 MHz, d_6-DMSO, 298 K)): ŭ (ppm) 190.6, 171.2, 148.3, 147.2, 144.4, 128.6, 124.4, 123.6, 118.9, 110.2, 78.2, 64.8. HRMS (ESI, -ve): calcd. for $C_{19}H_{13}N_4O_6S_2$ $[M-H]^-$ *m/z* = 457.0282, found 457.0287.

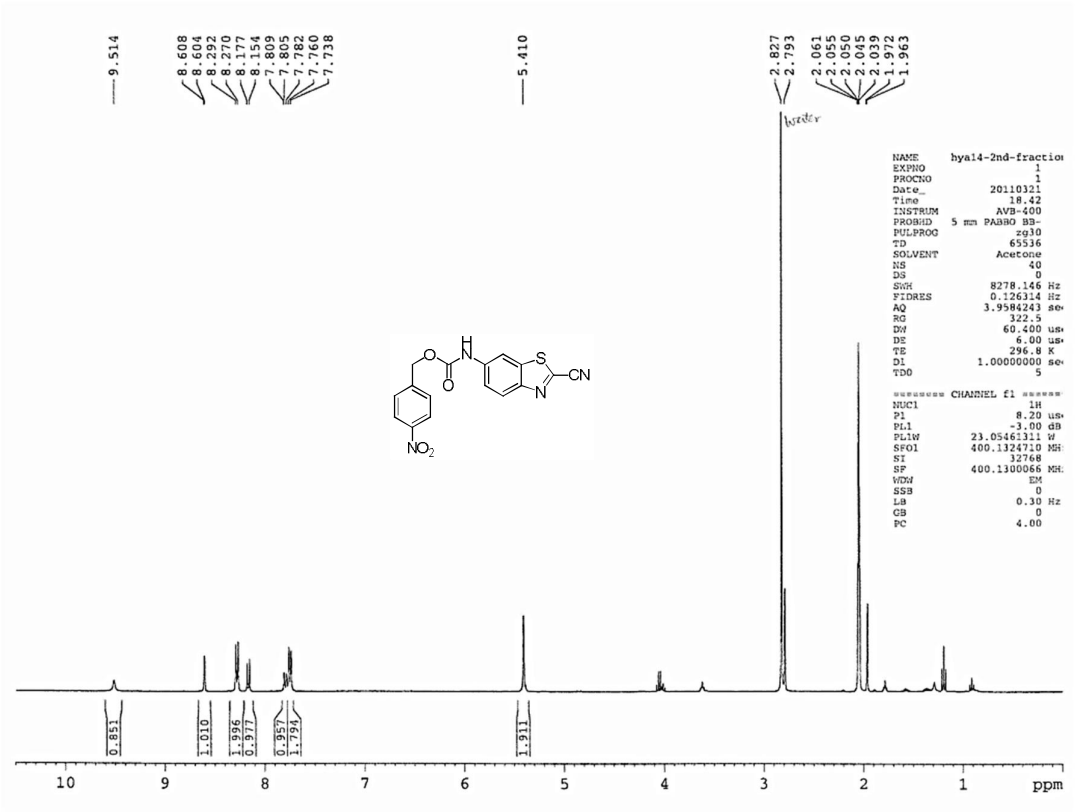

Figure S1. ^{1}H NMR (400 MHz, d_6-acetone, 298 K) spectrum of **1a**.

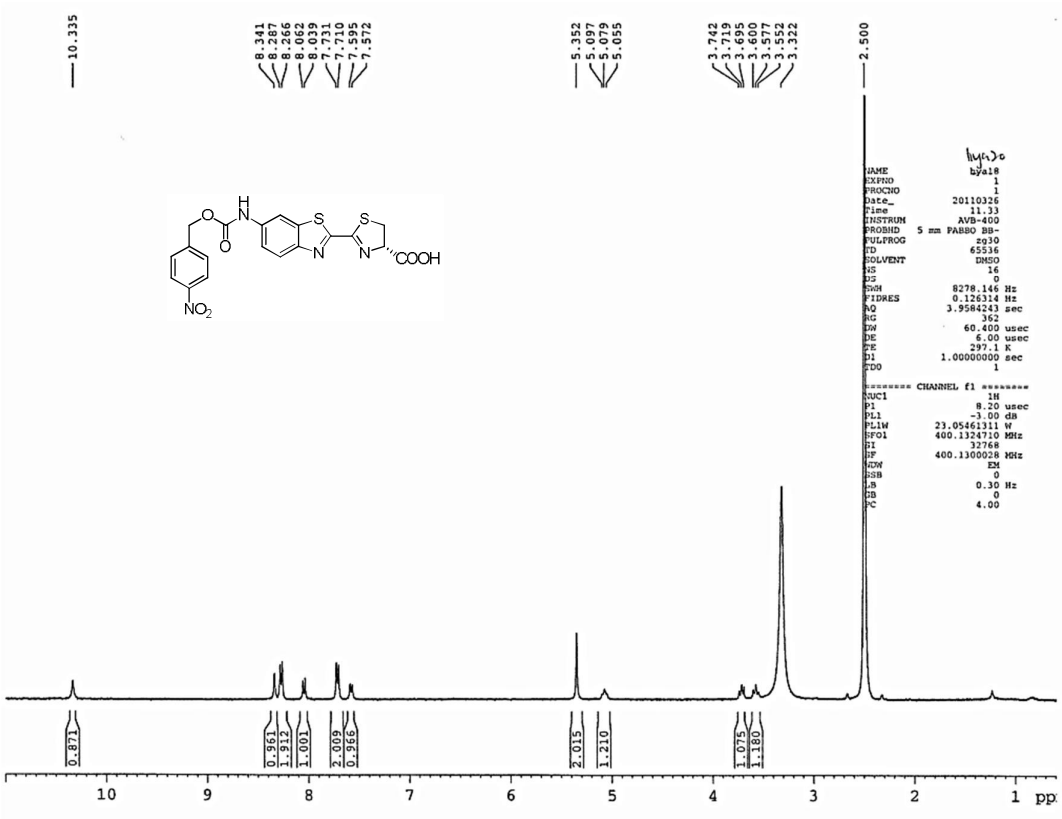

Figure S2. ^{1}H NMR (400 MHz, d_6-DMSO, 298 K) spectrum of **1**.

2. In vitro bioluminescent nitroreductase assays

Millipore water was used to prepare all aqueous solutions. Luciferase and nitroreductase (from *E. coli*) were purchased from Promega and Sigma-Aldrich respectively. All other chemicals were from commercial sources. Measurements of bioluminescence were performed in 50 mM Tris buffer at pH 7.4 at 37 °C and were recorded using a Molecular Device SpectraMax M2 plate reader. Samples for bioluminescent measurement were placed in white, opaque 96-well plates from Corning Inc. Bioluminescence was initiated by addition of a luciferase solution (100 μg/ml) in Tris buffer (50 mM, pH 7.4, 10 mM $MgCl_2$, 0.1 mM $ZnCl_2$ and 2 mM ATP). In a typical experiment, a 5 εM solution of probe **1** (100 εl) was incubated with either 1 unit of nitroreductase in the presence of 0.5 mM NADH or 0.1 mM of other reducing agents for 1 hour (or the specified time in the time course experiment) at room temperature. Equal volume of the luciferase solution was added to initiate bioluminescence and the bioluminescent signals were monitored for 1 hour.

3. Bacterial cell culture

Overnight culture of *Escherichia coli* ATCC25922 in Luria broth (LB) medium was used to inoculate (1% v/v) fresh LB medium. Similar procedure was carried out for *Bacillus subtilis* strain 168 except brain-heart infusion (BHI) medium was used for culture growth. Unless specified, after 2 hours incubation at 37°C, bacterial cells reaching mid-logarithm phase were harvested by centrifugation at 3000g for 3 minutes, washed twice and resuspended in ¼ of the original volume with Tris buffer (50 mM Tris, pH 7.4, 0.1 mM $ZnCl_2$, 0.1 mM $MgCl_2$). The cell suspension was then aliquoted and treated under different conditions. To measure nitroreductase activity, 10 εM of **1** was further incubated with bacterial cells for 1 hour at 37°C. Samples were then lysed by sonication, and clarified cell lysates were obtained by microcentrifugation at 12000g for 30s. Alternatively for non-lysis condition without sonication, supernatant was collected for measurement of nitroreductase activity. Grown bacteria were heat-killed by incubating at 85°C for 10 mins. Dicoumarol (reductase inhibitor) was used at 15 εM. OD_{600} measurement was done by using the NanoDrop1000.

4. *In vivo* measurement of nitroreductase activity

To measure nitroreductase activity in cell lysates, equal volume of Tris buffer containing 2 mM ATP and 100 εg/ml luciferase was added to lysates. Alternatively for whole cell detection, equal volume of Tris buffer containing 2 mM ATP and 100 εg/ml luciferase was added to the supernatant. Relative luminescence was measured in triplicate in white and opaque flat-bottomed 96-well plates using the DTX880 multimode detector to acquire luminescence.

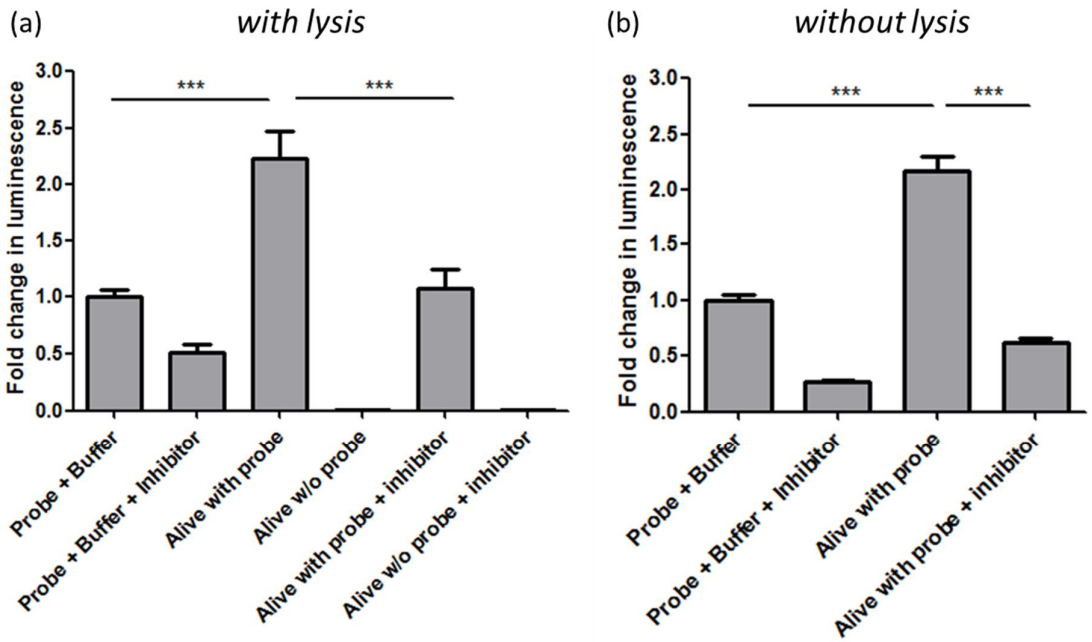

Figure S3. Relative luminescence intensity from (a) clarified cell lysate and (b) supernatant of *E. coli* after growing in LB medium for two hours, followed with or without heat-kill treatment and in the presence or absence of 15 ε M dicoumarol. Error bars indicate the s.e.m. of three samples. ***: p <0.0001.

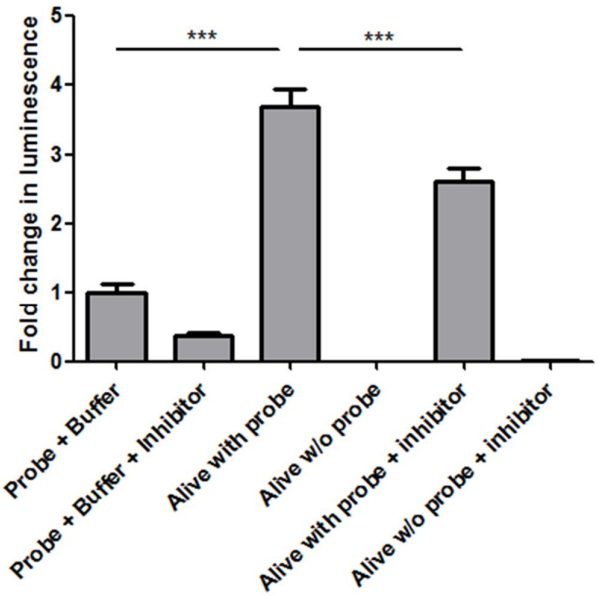

Figure S4. Relative luminescence intensity from clarified cell lysate of *B. subtilis* after growing in BHI medium for two hours, followed with or without heat-kill treatment and in the presence or absence of 15 ε M dicoumarol. Error bars indicate the s.e.m. of three samples. ***: p <0.0001.

5. Calculation of detection limit

Initially, 1 CFU was used to inoculate 2 ml of LB medium (0.5 CFU/ml). After 12 hrs of incubation, 1% grown culture (dilution factor = 100, 0.005 CFU/ml) was then used to inoculate fresh LB medium for subsequent detection analysis.

Assume t = 0 to t = 1 hour showed logarithmic growth, OD_{600} of 0.07 to 0.12 showed 2-fold increase of bioluminescent signal.

Hence, detection limit ≈ 0.005 x 0.12/0.07 ≈ 0.01 CFU/ml

To note, from t = 0 to t = 1 hour, as the growth curve is not linear, the above calculated detection limit underestimate the actual detection limit. This mathematical calculation demonstrates that the detection limit of our probe (0.01 CFU/ml) is approximately 5 times lower than the established protocol (0.05 CFU/ml, ref. 13 in the main text).