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Electronic Supplementary Information ESI

Increasing antibiotic activity by rapid bioorthogonal conjugation of drug to resistant bacteria using an upconverted light-activated photocatalyst

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S1. Experimental Section

S1.1. Materials

Yttrium (III) acetate hydrate ($Y(CH_3CO_2)_3.xH_2O$, 99.9%), ytterbium (III) acetate hydrate ($Yb(CH_3CO_2)_3.xH_2O$, 99.9%), erbium (III) acetate hydrate ($Er(CH_3CO_2)_3.xH_2O$, 99.9%), neodymium (III) acetate hydrate ($Nd(CH_3CO_2)_3.xH_2O$, 99.9%), oleic acid (OA, 65-88%), 1-octadecene (90%), ammonium fluoride (NH_4F, ≥ 98%), N-hydroxysuccinimide (NHS, 98%), N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC, commercial grade), 1-hydroxybenzotriazole hydrate (HOBT, 97%), 5-norbornene-2-endo-acetic acid, dibenzocyclooctyne-amine (DBCO-NH_2), diethylene glycol (≥ 99%), sodium citrate tribasic dihydrate (≥ 99%), chloramphenicol (bioreagent grade), vancomycin hydrochloride (pharmaceutical secondary standard) were purchased from Sigma-Aldrich. 4-oxo-4((6-(6-(pyridine-2-yl)-1,4-dihydro-1,2,4,5-tetrazin-3-yl)pyridine-3-yl)butanoic acid (dHTz-COOH **1a**) and 2-amino-*N*-(6-(6-(pyridin-2-yl)-1,4-dihydro-1,2,4,5-tetrazin-3-yl)pyridin-3-yl)acetamide (dHTz-NH_2) were purchased from Biocone Frontier Bio-chem Reagents. Methanol (HPLC grade), cyclohexane (HPLC grade) and dimethylformamide (DMF, HPLC grade) were purchased from Tedia. NaOH (> 99%) was purchased from Sinopharm Chemical Reagent Co. Ltd. Toluidine blue O (for microscopy) and N,N-diisopropylethylamine (DIPEA, for peptide synthesis, ~2 M in 1-methyl-2-pyrrolidinone) were purchased from Fluka. N,N,N′,N′-Ethylenediaminetetraacetic acid, disodium salt, dihydrate (EDTA, ≥ 99%) was purchased from USB Corporation. N,N,N′,N′-Tetramethyl-*O*-(1*H*-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU, 99.67%) was purchased from GL Biochem (Shanghai) Ltd. 3-azido-D-alanine HCl (ADA) was purchased from Jena Bioscience, and AFDye555-DBCO was purchased from Click Chemistry Tools. KCl (≥ 99.5%), KH_2PO_4 (≥ 99.5%), $Na_2HPO_4{\cdot}2H_2O$ (≥ 99.5%) were purchased from Merck KGaA. NaCl (99.9%) was purchased from VWR BDH Prolabo® Chemicals. All chemicals were used as received. Three bacterial strains were used; Van-resistant *Enterococcus faecium* (VanA genotype, ATCC 51559) and *Enterococcus faecalis* (VanB genotype, ATCC 51299), and Van-susceptible *B. subtilis* (ATCC 6051).

S1.2. Core UCNC synthesis

$Y(CH_3CO_2)_3$ (72.89 mg), $Yb(CH_3CO_2)_3$ (50.66 mg), $Er(CH_3CO_2)_3$ (0.763 mg) and $Nd(CH_3CO_2)_3$ (1.289 mg) were added into a vial and diluted with methanol (15 mL). The resulting solution was sonicated for 30 min and added into a three-neck round bottom flask along with oleic acid (3 mL) and 1-octadecene (7 mL). The mixture was heated to 100 °C to remove water and oxygen, and further heated to 150 °C for 1 h under N_2 environment. The

mixture was then cooled to room temperature. A methanol solution (6 mL) containing NaOH (1mmol) and NH_4F (1.6 mmol) was added and the mixture stirred for 30 min at 50 °C. Subsequently the mixture was heated to 100 °C under vacuum to remove methanol, oxygen and water before bringing the temperature slowly up to 300 °C and maintained for 2 h under N_2 atmosphere. The mixture was cooled to room temperature, washed twice with ethanol and centrifuged to retrieve the nanoparticles.

S1.3. Core-shell UCNC synthesis

$Y(CH_3CO_2)_3$ (95.77mg) and $Nd(CH_3CO_2)_3$ (12.89 mg) were first dissolved in methanol (18 mL) in a three-neck round bottom flask, followed by the addition of a cyclohexane solution containing core nanoparticles (2 mL), 1-octadecene (7 mL) and oleic acid (3 mL). The mixture was heated to 100 °C to remove water and oxygen, and further heated to 150 °C for 1 h under N_2 environment. The mixture was then cooled to room temperature. A methanol solution (6 mL) containing NaOH (1mmol) and NH_4F (1.6 mmol) was added and the mixture stirred for 30 min at 50 °C under N_2 environment. Subsequently the mixture was heated to 100 °C under vacuum to remove methanol, oxygen and water before bringing the temperature slowly up to 310 °C and maintained for 3 h under N_2 atmosphere. The mixture was then cooled to room temperature, washed twice with ethanol and centrifuged to retrieve the core-shell upconversion nanocrystals. All core and core-shell nanocrystals were characterized using TEM (JEM 1400, Jeol) and AFM (MultiMode 8, Bruker) and XRD (D8 Advance, Bruker).

S1.4. Ligand exchange of UCNC

A mixture of sodium citrate (2 mmol) and diethylene glycol (15 mL) was first heated to 110 °C under N_2 environment for 30 min. Core-shell UCNC in cyclohexane (2 mL) and toluene (4 mL) were added in to the mixture. The reaction mixture was heated to 160 °C under N_2 flow for 30 min to evaporate off the cyclohexane and toluene. The reaction mixture was further maintained at 160 °C for 3 h under N_2 environment before cooling to room temperature. The mixture was subsequently centrifuged, and the retrieved cit-capped core-shell UCNC washed three times with deionized water before dispersing in DI water (2 mL). The emission spectrum of cit-capped core-shell UCNC was recorded by exciting the sample using an 808 nm DPSS laser (VA-I-DC-808, Beijing Viasho Technology) and the spectrum recorded using an optical spectrometer (AvaSpec-ELS2048, Avantes).

S1.5. Linking toluidine blue O to cit-capped UCNC

Cit-capped core-shell UCNC and toluidine blue O (TB, 0.5 mg) were first added into DMF (600 μL). 50 μL of NHS in DMF (40 mM), 100 μL of EDC in DMF (40 mM) and 50 μL of HOBT in DMF (40 mM) were added into the DMF mixture of TB and cit-capped UCNC. The reaction mixture was stirred for 4 h at room temperature. Following that, the TB-UCNC were retrieved by centrifugation and washed three times with deionized water. Dynamic light scattering (DLS, Zetasizer Nano ZPS, Malvern) was also used to estimate the hydrodynamic diameter of a sphere with the same diffusion coefficient as the corresponding nanocrystal: i) as-prepared OA-UCNC in cyclohexane (~ 25 mg mL^{-1}), ii) cit-capped UCNC in DI water (~ 25 mg mL^{-1}) and iii) TB-UCNC in DI water (~ 25 mg mL^{-1}).

S1.6. Synthesis of $NaYF_4$@$NaYF_4$ nanoparticles

$Y(CH_3CO_2)_3$ (106.42 mg) was added into a vial and diluted with methanol (15 mL). The resulting solution was sonicated for 30 min and added into a three-neck round bottom flask along with oleic acid (3 mL) and 1-octadecene (7 mL). The mixture was heated to 100 °C to remove water and oxygen, and further heated to 150 ºC for 1 h under N_2 environment. The mixture was then cooled to room temperature. A methanol solution (6 mL) containing NaOH (1mmol) and NH_4F (1.6 mmol) was added and the mixture stirred for 30 min at 50 °C. Subsequently the mixture was heated to 100 °C under vacuum to remove methanol, oxygen and water before bringing the temperature slowly up to 300 °C and maintained for 2 h under N_2 atmosphere. The mixture was cooled to room temperature, washed twice with ethanol and centrifuged to retrieve the $NaYF_4$ nanoparticles.

$Y(CH_3CO_2)_3$ (106.42mg) was first dissolved in methanol (18 mL) in a three-neck round bottom flask, followed by the addition of a cyclohexane solution containing $NaYF_4$ nanoparticles (2 mL), 1-octadecene (7 mL) and oleic acid (3 mL). The mixture was heated to 100 °C to remove water and oxygen, and further heated to 150 ºC for 1 h under N_2 environment. The mixture was then cooled to room temperature. A methanol solution (6 mL) containing NaOH (1mmol) and NH_4F (1.6 mmol) was added and the mixture stirred for 30 min at 50 °C under N_2 environment. Subsequently the mixture was heated to 100 °C under vacuum to remove methanol, oxygen and water before bringing the temperature slowly up to 310 °C and maintained for 3 h under N_2 atmosphere. The mixture was then cooled to room temperature, washed twice with ethanol and centrifuged to retrieve the $NaYF_4$@$NaYF_4$ nanocrystals.

S1.7. Synthesis of Nb-DBCO

DBCO-NH_2 (28 mg), 5-norbornene-2-endo-acetic acid (16 mg), HBTU (40 mg) and DIPEA (10 μL) were dissolved in DMF (500 μL) and stirred for 4 h at room temperature. Nb-DBCO was isolated by reversed-phase HPLC (Waters 2695 Separations Module) and lyophilized. NMR spectroscopy was carried out on an NMR spectrometer (Ultrashield 400 Plus, Bruker). Liquid chromatography-mass spectrometry (LCMS) was measured using a LCMS spectrometer (UltiMate 3000, Thermo Scientific). The ^{1}H NMR spectra of 5-norbornene-2-endo-acetic acid, DBCO and Nb-DBCO are presented in Fig. S20 and S21. The LCMS spectrum of Nb-DBCO is presented in Fig. S22.

The Nb-DBCO was identified by the MS peak at *m*/*z* 411.27. Calculated $[M+H]^+$: 411.52.

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.67 – 7.56 (m, 2H), 7.54 – 7.43 (m, 3H), 7.42 – 7.38 (m, 1H), 7.38 – 7.26 (m, 2H), 6.09 (dt, *J* = 5.0, 2.1 Hz, 1H), 5.33 (t, *J* = 4.8 Hz, 1H), 5.05 (d, *J* = 14.0 Hz, 1H), 3.09 (dt, *J* = 13.5, 7.0 Hz, 2H), 2.91 (dd, *J* = 13.2, 6.5 Hz, 1H), 2.58 (d, *J* = 3.9 Hz, 1H), 2.18 (t, *J* = 7.3 Hz, 2H), 1.98 (d, *J* = 6.1 Hz, 2H), 1.24 (d, *J* = 5.2 Hz, 10H), 0.86 (s, 2H).

S1.8. Synthesis of Van-dHTz

dHTz-NH_2 (4 mg), Van (16 mg), HBTU (8 mg) and DIPEA (10 μL) were dissolved in DMF (100 μL) and stirred for 4 h at room temperature. The Van-dHTz was purified by reversed-phase HPLC and lyophilized. The ^{1}H NMR spectra of dHTz-NH_2, Van and Van-dHTz are presented in Fig. S23 and S24. The LCMS spectrum of Van-dHTz is presented in Fig. S25.

The Van-dHTz was identified by the MS peak at *m*/*z* 871.06. Calculated $[M/2+H]^+$: 871.785.

^{1}H NMR (400 MHz, Deuterium Oxide) δ 8.84 – 8.68 (m, 1H), 8.60 (dt, *J* = 4.7, 1.3 Hz, 1H), 8.08 (dd, *J* = 8.7, 2.5 Hz, 1H), 8.03 – 7.88 (m, 2H), 7.75 (s, 1H), 7.65 – 7.42 (m, 2H), 7.30 (s, 1H), 7.14 (s, 1H), 6.47 (t, *J* = 2.5 Hz, 1H), 5.68 (s, 1H), 5.52 – 5.21 (m, 3H), 4.48 (s, 1H), 4.21 (s, 1H), 3.98 (s, 1H), 3.74 (s, 1H), 3.58 (s, 1H), 3.39 (s, 1H), 3.29 (s, 1H), 2.72 (d, *J* = 7.9 Hz, 2H), 2.63 (p, *J* = 2.0 Hz, 2H), 2.01 (d, *J* = 12.0 Hz, 1H), 1.38 (s, 1H), 1.13 (d, *J* = 6.2 Hz, 1H), 0.88 (dd, *J* = 14.4, 6.2 Hz, 3H).

S1.9. ATR-FTIR measurements

OA-capped core-shell UCNC, cit-capped core-shell UCNC, and TB-UCNC were dried overnight in an oven, and separately deposited onto an ATR crystal. The ATR-FTIR spectra were measured using an FTIR spectrometer (Spectrum 100, PerkinElmer).

S1.10. Luminescence lifetime measurements

The time-resolved luminesce lifetime decay profiles of cit-capped UNCN and TB-UCNC were recorded using a fluorescence lifetime microscope (PicoQuant, MicroTime 200) equipped with an inverted microscope (IX71, Olympus) and a scanning stage (P-733.2CL, Physik Instruments). Pulsed laser excitation was generated from an 808 nm CW laser (MDL-H-808-2.5W, Changchun New Industries Optoelectronics Technology Co., Ltd) using a dual channel DDS signal generator/counter (MHS-5200A-2.5 MHz, EINST Technology Pte. Ltd) and the frequency of the pulsed laser is 1000 Hz. After passing the pulsed laser light through an excitation filter (TECHSPEC Bandpass Filter 810/10 nm, Edmund Optics), circularly polarized excitation light was achieved by using half-wave and quarter-wave plates before focusing through an air objective lens (20×, N.A. 0.4, Olympus). The emission was then passed through a dichroic mirror (ZT720sp 25.5 x 36 x 1 mm, Chroma), notch filter (TECHSPEC OD4 808 nm, Edmund Optics) and emission filter (HQ685/70M) before being detected by a single-photon avalanche diode detector (SPAD, SPCM-AQR-15, PerkinElmer).

S1.11. UCNC quantum yield measurement

OA-capped core-shell UCNC in cyclohexane (20 mg mL^{-1}) and $NaYF_4$@$NaYF_4$ nanocrystals in cyclohexane (20 mg mL^{-1}) were first prepared. The quantum yield of the OA-capped UCNC was calculated using the equation: Q_0 = (photons emitted/photons absorbed), where photons emitted is the emission intensity of the OA-capped core-shell UCNC.[R3] Photons absorbed = *P*[*reference*] – *P*[*sample*], where *P*[*reference*] and *P*[*sample*] are intensities of the excitation light after passing through the $NaYF_4$@$NaYF_4$ nanoparticle solution and OA-capped core-shell UCNC solution, respectively. The samples were excited using an 808 nm DPSS laser (VA-I-DC-808, Beijing Viasho Technology) and the light detected using a spectrometer (AvaSpec-ELS2048, Avantes).

S1.12. Estimating the number of TB molecules linked to a cit-capped UCNC

A series of TB in PBS buffer with known concentrations (1.0, 2.0, 4.0, 8.0, 10.0 μg mL^{-1}) were prepared. Reversed-phase HPLC experiments were performed to obtain a calibration curve

based on the peak area at 650 nm. 10 μL of the stock TB-UCNC solution was diluted to 1 mL and HPLC measurement was conducted to determine the mass of TB linked to the UCNC. The mass of TB was determined to be 0.541 μg. 10 μL of the same stock solution of TB-UCNC was dried in an oven to obtain the mass of TB-UCNC. The determined mass of UCNC was 89.549 μg.

The volume of a hexagonal unit cell is given by: $V_{cell} = (\sqrt{3}/2)a^2c = 0.1086$ nm^3, where the lattice parameters for hexagonal unit cells a and c are 5.96 Å and 3.53 Å, respectively.[R1,R2] Assume that the core nanoparticle is a sphere with an average diameter d = 29.4 nm, and the core-shell nanocrystal is a cylinder with an average length and width of l = 59.5 nm and w = 31.7 nm, respectively (see Fig. 1A and B) . The volumes of the core, core-shell and shell are $V_c = (4/3)\pi(d/2)^3 = 13299$ nm^3, $V_{cs} = \pi(w/2)^2 l = 46936$ nm^3 and $V_s = V_{cs} - V_c = 33637$ nm^3, respectively. The number of unit cells in a core is estimated by: $n_c = V_c/V_{cell}$ =122459, and the number of unit cells in a shell is estimated by: $n_s = V_s/V_{cell} = 309733$.

Given that the atomic weight of Na AW_{Na} = 22.989 g mol^{-1}, F AW_F = 18.998 g mol^{-1}, Y AW_Y = 88.905 g mol^{-1}, Nd AW_{Nd} =144.242 g mol^{-1}, Yb AW_{Yb} = 173.054 g mol^{-1} and Er AW_{Er} = 167.259 g mol^{-1}. The atomic weight of a single hexagonal unit cell of the core ($NaYF_4$:Nd^{3+}/Yb^{3+}/Er^{3+}) is calculated by: $AW_c = (1.5 \times AW_{Na}) + (6 \times AW_F) + 1.5 \times (0.685 \times AW_Y + 0.01 \times AW_{Nd} + 0.3 \times AW_{Yb} + 0.005 \times AW_{Er}) = 321.11$ g mol^{-1}.[R2] The atomic weight of a single hexagonal unit cell of the shell ($NaYF_4$:Nd^{3+}) is calculated by: $AW_s = (1.5 \times AW_{Na}) + (6 \times AW_F) + 1.5 \times (0.9 \times AW_Y + 0.1 \times AW_{Nd}) = 290.13$ g mol^{-1}. The molecular weight of the core-shell UCNC $MW_{cs} = (n_c \times AW_c) + (n_s \times AW_s) = 1.29 \times 10^8$ g mol^{-1}. From the determined mass of TB and UCNC, the number of TB molecules linked to a UCNC is 2548.

S1.13. Conversion of dHTz 1a to Tz 1b

PBS buffer was prepared by adding the following to deionized water: NaCl (8 g L^{-1}), KCl (0.2 g L^{-1}), Na_2HPO_4 (1.42 g L^{-1}), KH_2PO_4 (0.24 g L^{-1}) and EDTA (0.672 g L^{-1}), and the pH adjusted to 7.4 using 1 M NaOH solution. The TB-UCNC was first washed with PBS buffer (1 mL) twice before dispersing in PBS buffer (0.5 mL) for further experiments. A stock solution of dHTz **1a** was first prepared. 0.5 mL of the stock dHTz **1a** solution was added into 0.5 mL PBS, and the absorption spectrum of dHTz **1a** was recorded using a UV-visible spectrometer (Cary 100, Varian). A mixture of dHTz **1a** (0.5 mL) and TB-UCNC in PBS buffer (0.5 mL) was stirred for 5 min under the irradiation of an 808 nm laser (4.3 W cm^{-2}) (MDL-H-808nm-2.5W, Changchun New Industries Optoelectronics Technology Co., Ltd). The mixture was

subsequently centrifuged at 10000 rpm for 10 min to remove TB-UCNC, and the absorption spectrum of the supernatant measured. Various amounts of TB-UCNC were used ranging from 0 to 30 mg mL^{-1}. A similar experiment was performed whereby a thin (1.5 mm) slice of chicken breast meat was place between the bacterial solution and laser source during light exposure. In this case, the amount of TB-UCNC used was 25.0 mg mL^{-1}.

The following control experiments were also performed. Three separate solutions were prepared: (i) a PBS solution of dHTz **1a**, (ii) a PBS solution of dHTz **1a** and TB (29.4 μM), and (iii) a PBS solution of dHTz **1a** and cit-capped UCNC (25.0 mg mL^{-1}). These solutions were irradiated with 808 nm light (4.3 W cm^{-2}) for 5 min, and the absorption spectra of the solutions recorded. A fourth solution containing dHTa **1a** and TB-UCNC (25.0 mg mL^{-1}) was kept in the dark for 5 min before removing the TB-UCNC by centrifugation. The absorption spectrum of the supernatant was then recorded.

S1.14. Conversion of Van-dHTz to Van-Tz

A Van-dHTz solution in PBS buffer with OD ~ 0.6 at 284 nm was prepared. The Van-dHTz solution was then mixed with TB-UCNC (25.0 mg mL^{-1}), and irradiated with 808 nm light (4.3 W cm^{-2}) for 5 min under continuous stirring. The solution was centrifuged to remove the TB-UCNC, and the supernatant collected for UV-vis absorption measurement.

S1.15. Uptake of ADA by bacteria

B. subtilis, VanA and VanB bacterial cells were seeded and grown on Lysogeny broth and shaken overnight (OD_{600} ~ 0.9, 0.83 and 0.85 for *B. subtilis*, VanA and VanB). ADA (10 mM) was added to the bacterial sample and incubated at 37°C for 1 h to allow for the metabolic uptake of ADA onto bacterial peptidoglycan. Subsequently, the medium was removed and bacteria recovered by centrifugation. The bacteria was then washed with PBS buffer (pH 7.4), re-suspended in PBS buffer and incubated for 1 h with DBCO functionalized AFDye555 (AFDye555-DBCO). The bacteria with AFDye555 dye functionalized on the cell surface were washed with PBS buffer before deposition onto glass coverslips and visualized using a confocal microscope (LSM 800, Carl Zeiss). The excitation wavelength was 561 nm and the emission wavelength range monitored was from 565 nm to 630 nm.

S1.16. DBCO-Nb and TB-UCNC toxicity experiments

B. subtilis, VanA and VanB bacteria were seeded and grown on Lysogeny broth and shaken overnight. ADA (10 mM) was added to the bacterial sample and incubated at 37°C for 1 h to

allow for the metabolic uptake of ADA. Subsequently, the medium was removed and bacteria recovered by centrifugation. The bacteria was then washed with PBS buffer (pH 7.4), re-suspended in PBS buffer and incubated for 1 h with Nb-DBCO (10 mM). After washing the bacteria whose cell walls are functionalized with Nb with PBS buffer, the cells were re-suspended in Lysogeny broth and shaken for 3 h before being plated on a LB agar. The number of colony forming units (CFU) was counted after 12 h incubation at 37 °C.

To assess the toxicity of TB-UCNC, bacteria cells were seeded and grown in Lysogeny broth and shaken overnight. TB-UCNC (25.0 mg mL^{-1}) was added into the bacterial solution and irradiated with an 808 nm laser for 5 min under continuous stirring. The solution was further shaken for 3 h before plated on a LB agar. The number of CFU was counted after 12 h incubation at 37 °C.

S1.17. MIC of Van against *B. subtilis*, VanA, VanB

B. subtilis, VanA, VanB bacterial cells were seeded and grown in Lysogeny broth and shaken overnight. The bacterial solution was diluted 5-times with Lysogeny broth and then incubated for another 3 h. Various concentrations of Van (100 μL) were separately added into the bacterial solution (100 μL) and shaken for 3 h. 10 μL of a 100-times diluted bacterial solution was then applied onto LB agar plates and the concentration of Van that resulted in 90 % bacterial growth inhibition was taken to be the MIC value.

S1.18. MIC of Van-dHTz against *B. subtilis*, VanA and VanB

B. subtilis, VanA, VanB bacteria cells were seeded and grown on Lysogeny broth and shaken overnight. ADA (10 mM) was added to the bacterial solution and incubated at 37°C for 1 h to allow for the metabolic uptake of ADA onto bacterial peptidoglycan. Subsequently, the bacteria were recovered by centrifugation and washed in PBS buffer. After washing, the bacteria were re-suspended in PBS buffer and incubated for 1 h with Nb-DBCO (10mM). The Nb-functionalized bacteria were recovered by centrifugation and washed using PBS buffer. Various concentrations of Van-dHTz and TB-UCNC (25.0 mg mL^{-1}) were added into the bacterial solution and irradiated with 808 nm light (4.3 W cm^{-2}) for 5 min. The bacteria were recovered by centrifugation, washed using PBS buffer, re-suspended in Lysogeny broth and incubated for another 3 h before being plated on an LB agar. The CFU values and MIC were determined. For VanB, a similar experiment was performed whereby a thin (1.5 mm) slice of chicken breast meat was place between the bacterial solution and laser source during light exposure.

S1.19. Effect of NIR light irradiation

B. subtilis, VanA, VanB bacteria cells were seeded and grown on Lysogeny broth and shaken overnight. ADA (10 mM) was added to the bacterial solution and incubated at 37°C for 1 h to allow for the metabolic uptake of ADA onto bacterial peptidoglycan. Subsequently, the bacteria were recovered by centrifugation and washed with PBS buffer. After washing, the bacteria were re-suspended in PBS buffer and incubated for 1 h with Nb-DBCO (10 mM). The Nb-functionalized bacteria were recovered by centrifugation and washed using PBS buffer. Nb-functionalized bacteria were mixed and stirred with Van-dHTz (concentrations of Van are 0.09, 27.1, 9.6 μg mL^{-1} for *B. subtilis*, VanA and VanB, respectively) and TB-UCNC (25.0 mg mL^{-1}) while being exposed to 808 nm light (4.3 W cm^{-2}) for 5 min. Unbound Van-dHTz/Van-Tz was removed by centrifugation, and the bacteria washed with PBS buffer before re-suspension in Lysogeny broth. The bacterial cells were incubated at 37 ºC for 3 h before platted on a LB agar. In a control experiment, the above procedure was repeated in the absence of NIR light irradiation.

S.1.20. Photothermal effect

The temperature change profile of cit-capped UCNC suspended in PBS buffer (25 mg in 1 mL) was obtained by monitoring the time-resolved temperature change using a thermal imaging camera (FLIR Systems, Inc.). The solution was exposed to 808 nm light (4.3 W cm^{-2}) for 5 min and then the light source was turned off. Three laser 'on'/'off' cycles were performed.

B. subtilis, VanA, VanB bacteria were seeded and grown on Lysogeny broth and shaken overnight. Cells mixed with cit-UCNC (25 mg mL^{-1}, 1 mL) were irradiated with 808 nm light (4.3 W cm^{-2}) for 5 min. 10 μL of 1000× diluted bacterial solution was plated on an LB agar and incubated overnight. A control experiment was also performed where bacteria in the absence of cit-capped UCNC were irradiated by 808 nm light for 5 min, and the diluted (1000×) solution plated on an LB agar before overnight incubation.

S1.21. Conjugation efficiency of Van-dHTz to bacteria

B. subtilis, VanA, VanB bacteria were seeded and grown on Lysogeny broth and shaken overnight (OD_{600} ~ 0.8 – 0.9). ADA (10 mM) was added to the bacterial solution and incubated at 37°C for 1 h to allow for the metabolic uptake of ADA onto bacterial peptidoglycan. Subsequently, the bacteria were recovered by centrifugation and washed in PBS buffer. After washing, the bacteria were re-suspended in PBS buffer and incubated for 1 h with Nb-DBCO

(10 mM). The Nb-functionalized bacteria were recovered by centrifugation and washed using PBS buffer. A mixture of bacteria, Van-dHTz (*e.g.*, concentrations of Van for 1 set of experiment were 0.093, 17.9 and 6.1 μM for *B. subtilis*, VanA and VanB, respectively) and TB-UCNC (25 mg mL^{-1}) was irradiated with 808 nm light for 5 min. The mixture was centrifuged, and the supernatant containing free Van-dHTz was retrieved. The bacteria were washed once with PBS buffer solution, centrifuged and the supernatant collected. The concentration of unbound Van-dHTz in the combined supernatant was determined using UV-visible absorption spectroscopy with an extinction coefficient of ε_{284} = 4.8 × 10^4 M^{-1} cm^{-1} at 284 nm; as determined from a calibration curve and Beer Lambert Law.

S1.22. Intracellular accumulation of UDPMurNac-pp

B. subtilis, VanA, VanB bacteria cells were seeded and grown on Lysogeny broth and shaken overnight. Bacterial cell concentration was adjusted to OD_{600} 0.6 and incubated with chloramphenicol (130 μg mL^{-1}) for 15 min. For each bacteria, three samples were prepared. The first was untreated bacteria. The second was bacterial solution with Van (0.11, 27.0 and 8.9 μg mL^{-1} for *B. subtilis*, VanA and VanB, respectively) added and incubated for 1 h before removal of Van by centrifugation. The third was Nb-functionalized bacterial solution with Van-dHTz (Van concentrations from 1 set experiment were 0.11, 27.0 and 8.9 μg mL^{-1} for *B. subtilis*, VanA and VanB, respectively) added followed by 808 nm light (4.3 W cm^{-2}) irradiation for 5 min in the presence of TB-UCNC (25.0 mg mL^{-1}). Unbound Van-dHTz/Van-Tz was removed by centrifugation and the bacterial solution incubated for 1 h. The bacteria were washed with DI water and transferred into boiling water and treated for 30 min. The cell extract after the boiling water treatment was then retrieved by centrifugation. The supernatant's pH was adjusted to 2.0 with phosphoric acid and DI water. The UDPMurNac-pp was isolated by HPLC and identified by LCMS. The absorbance peak of UDPMurNac-pp at 260 nm was measured using an absorption spectrometer for the three bacterial strains and compared.

S2. Supplementary Figures

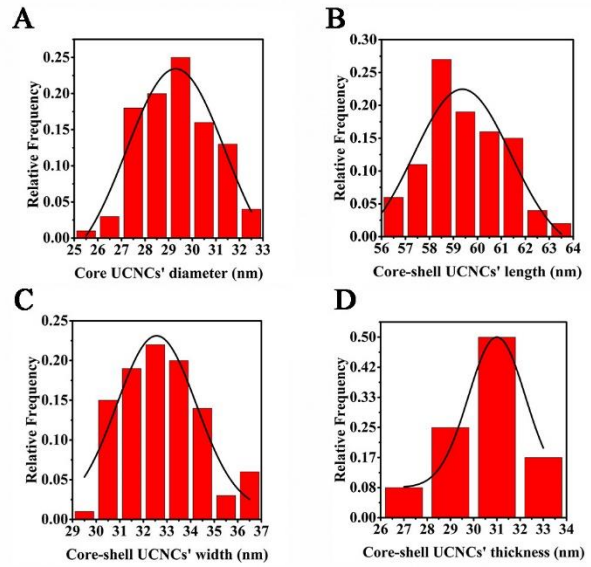

Fig. S1 Histograms of the size distribution of the as-prepared core upconversion nanoparticles and core-shell upconversion nanocrystals. (A) Histogram of the diameter of the as-prepared core. The Gaussian fit gives an average diameter of 29.4 ± 1.6 nm. (B) Histogram of the length of the as-prepared core-shell UCNC. The Gaussian fit gives an average length of 59.5 ± 1.6 nm. (C) Histogram of the width of the as-prepared core-shell UPNC. The Gaussian fit gives an average width of 32.8 ± 1.7 nm. (D) Histogram of the height of the as-prepared core-shell UCNC. The Gaussian fit gives an average height of 30.5 ± 1.4 nm. (A)-(C) are based on 100 nanocrystals, and (D) is based on 12 nanocrystals.

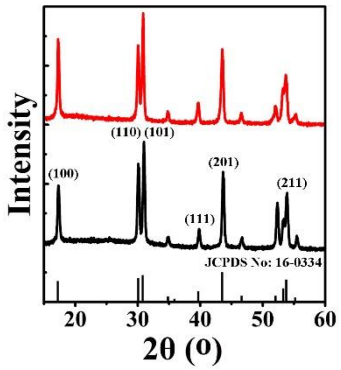

Fig. S2 XRD patterns of core $NaYF_4$: $Nd^{3+}/Yb^{3+}/Er^{3+}$ nanoparticle (black) and core-shell $NaYF_4$:$Nd^{3+}/Yb^{3+}/Er^{3+}$@$NaYF_4$:Nd^{3+} nanocrystals (red). The peaks for both the core nanoparticles and core-shell nanocrystals are well indexed to the peaks of standard hexagonal phase β-$NaYF_4$ (JCPDS No. 16-0334).

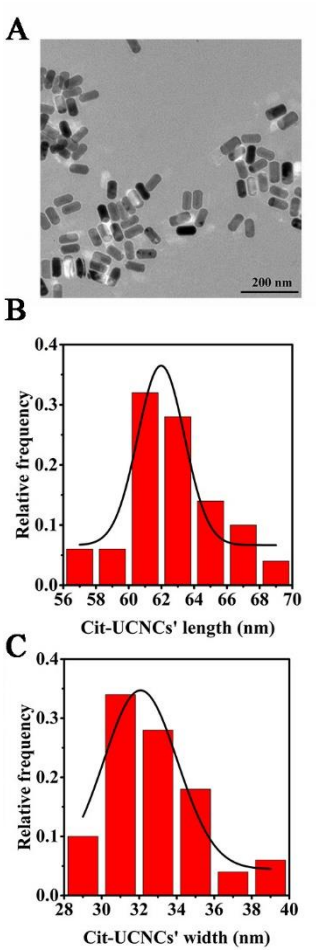

Fig. S3 (A) TEM image of cit-capped core-shell UCNC after ligand exchange. (B) Histogram of the length of the cit-capped core-shell UCNC. The Gaussian fit gives an average length of 62.7 ± 2.7 nm. C) Histogram of the width of the cit-capped core-shell UPNC. The Gaussian fit gives an average width of 32.8 ± 2.4 nm.

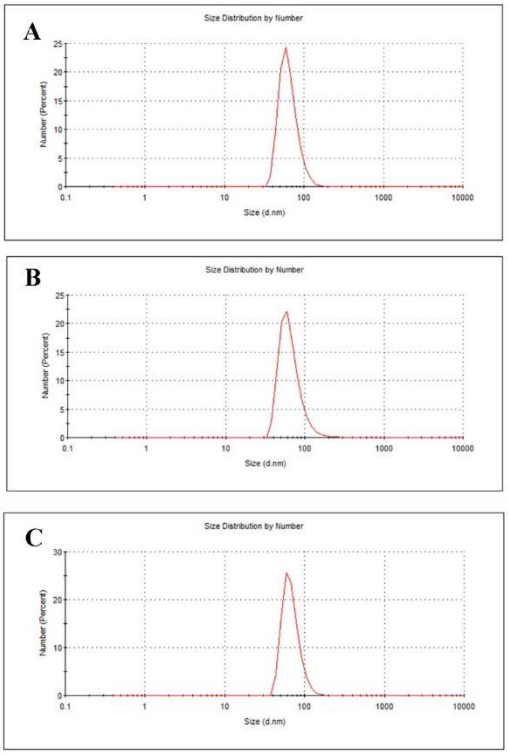

Fig. S4 Size distribution of OA-capped core-shell UCNC (A), cit-capped UCNC (B) and TB-UCNC (C) obtained by using the DLS technique.

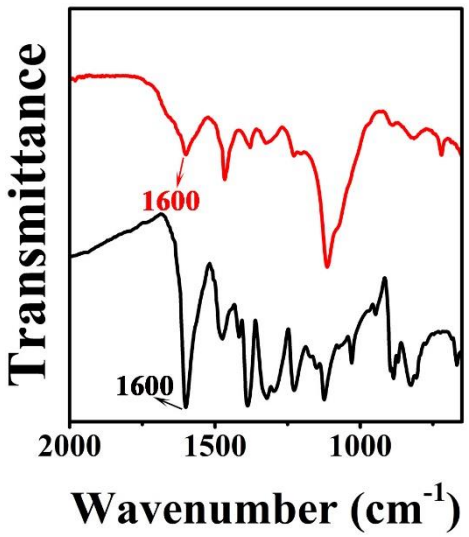

Fig. S5 ATR-FTIR spectra of TB-UCNC (red) and TB (black). The intense 1600 cm^{-1} peak observed for both TB-UCNC and TB arises from the dye's aromatic rings vibrational modes.[R4] This peak is not present in the ATR-FTIR spectrum of cit-capped UCNC (Fig. 1D).

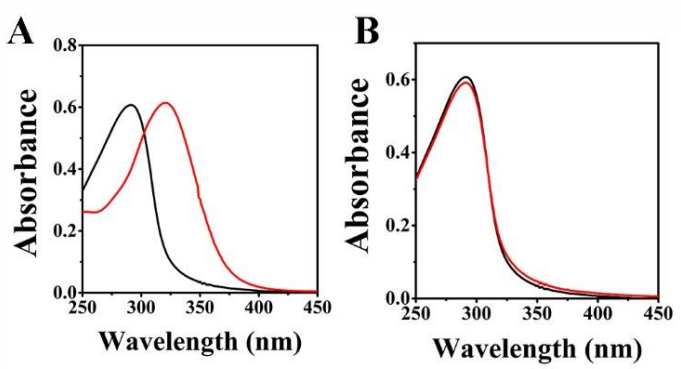

Fig. S6 (A) UV-vis absorption spectra of **1a** before (black) and after (red) exposure to 633 nm light (Melles Griot HeNe laser) for 5 min in the presence of TB (29.4 μM). (B) UV-vis absorption spectra of **1a** before (black) and after (red) exposure to 808 nm light for 5 min in the presence of TB.

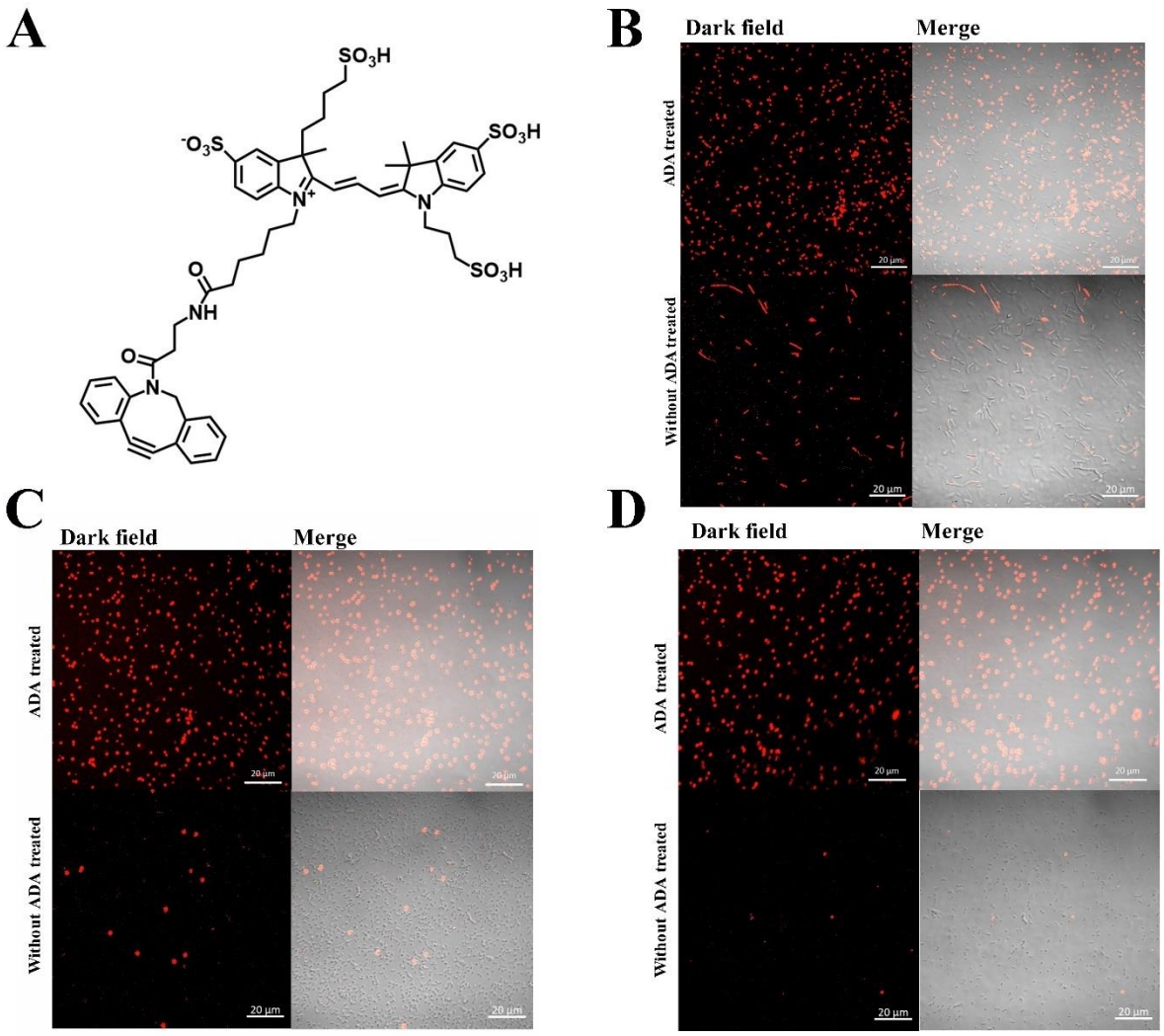

Fig. S7 (A) Chemical structure of AFDye555-DBCO (B) Confocal fluorescence microscopy images (dark field and merged dark field and bright field images) of azido-D-Alanine (ADA)-treated *B. subtilis* (top panels) and non-treated bacteria (bottom panels) mixed with AFDye555-DBCO. (C) Confocal fluorescence microscopy images (dark field and merged dark field and bright field images) of ADA-treated VanA (top panels) and non-treated bacteria (bottom panels) mixed with AFDye555-DBCO. (D) Confocal fluorescence microscopy images (dark field and merged dark field and bright field images) of ADA-treated VanB (top panels) and non-treated bacteria (bottom panels) mixed with AFDye555-DBCO. (λ_{ex}= 561 nm, λ_{em}= 565-630 nm)

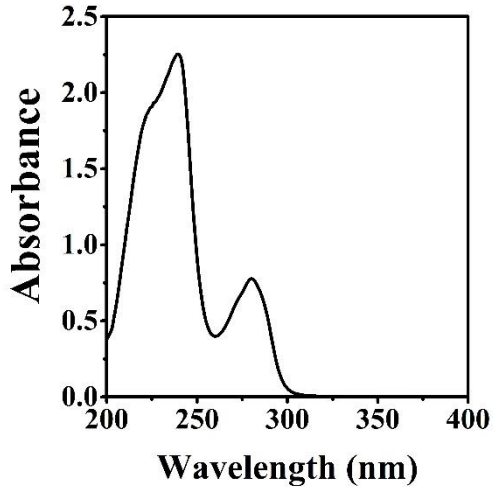

Fig. S8 Absorption spectrum of Van in PBS buffer (conc. 116.4 μM). Two peaks at 240 and 280 nm are observed.

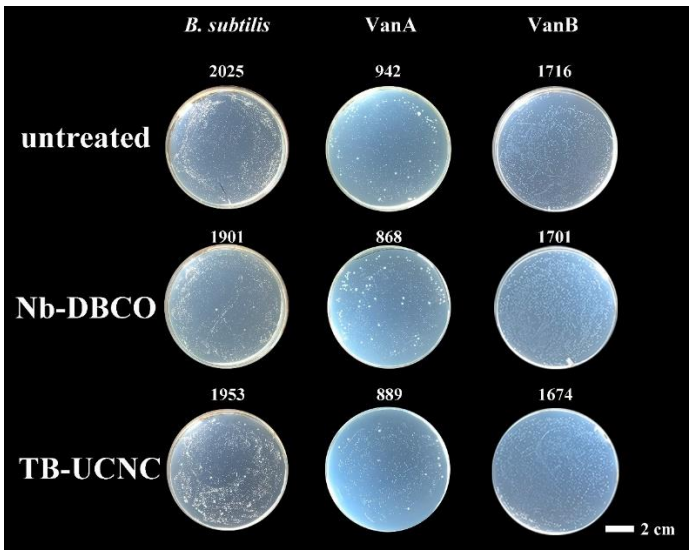

Fig. S9 Bacterial colonies of *B. subtlis* (first column), VanA (second column) and VanB (third column) on agar plates for untreated bacteria (top row), bacteria treated with ADA to form Nb-functionalized bacteria (middle row), and bacteria exposed to TB-UCNC and 808 nm light irradiation (5 min). The CFU values are presented above each plate.

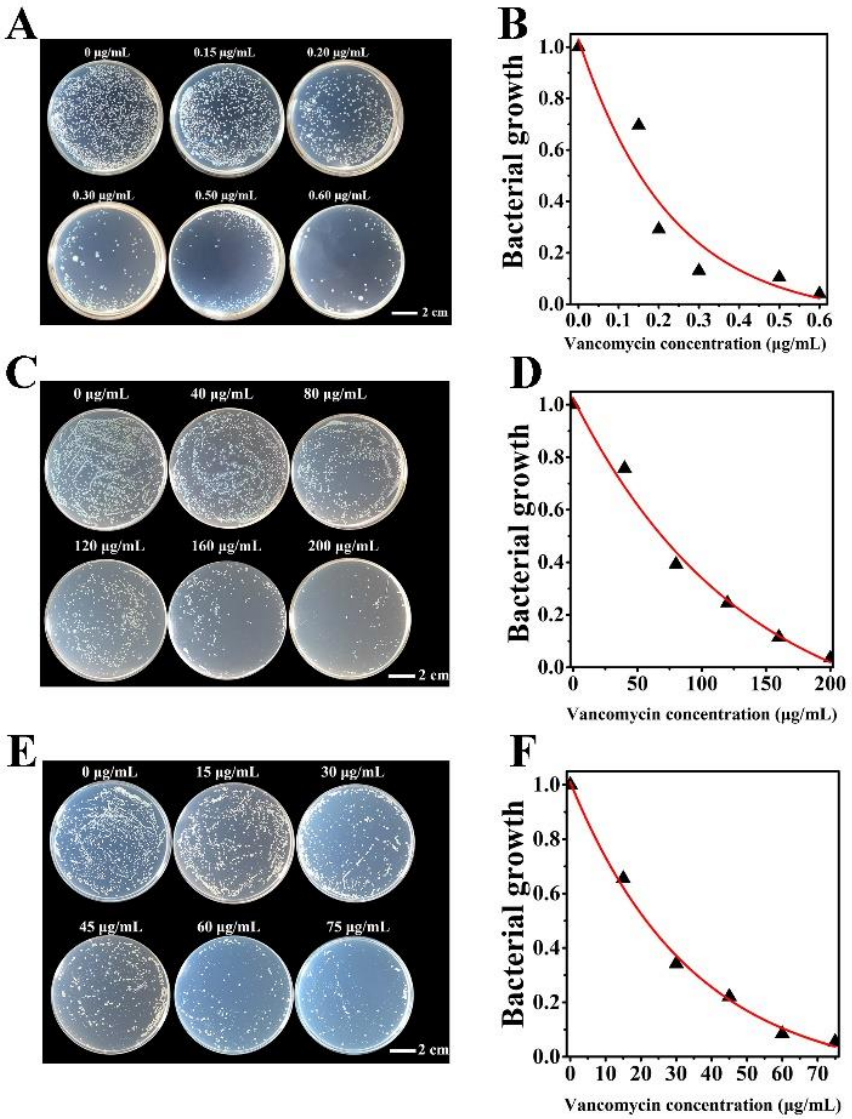

Fig. S10 Bacteria colonies of *B. subtilis* (A), VanA (C) and VanB (E) on agar plates after treatment with various concentrations of Van. Relative bacterial growth of *B. subtilis* (B), VanA (D) and VanB (F) after treatment with various concentrations of Van with respect to the growth in the absence of Van. The data points were fit to a single-exponential decay function, and the minimum concentrations of Van needed to give 90% growth inhibition (*i.e.*, MIC) are given in Table 1.

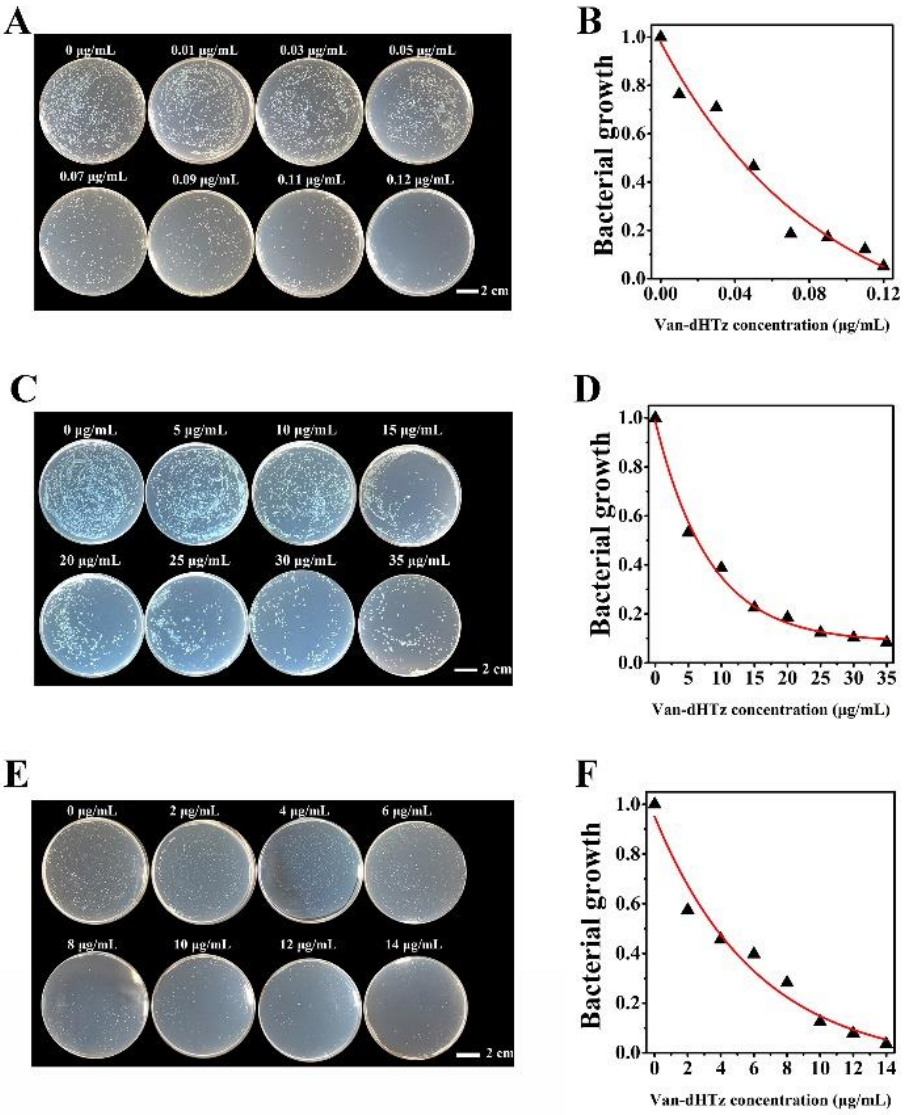

Fig. S11 Bacteria colonies of *B. subtilis* (A), VanA (C) and VanB (E) on agar plates after treatment with various concentrations of Van-dHTz and 808 nm light irradiation (5 min). Relative bacterial growth of *B. subtilis* (B), VanA (D) and VanB (F) after treatment with various concentrations of Van-dHTz with respect to the growth in the absence of Van. The data points were fit to a single-exponential decay function, and the MIC value taken to be the minimum concentrations of Van needed to give 90 % growth inhibition. The MIC values reported in Table 1 are based on the concentration of Van.

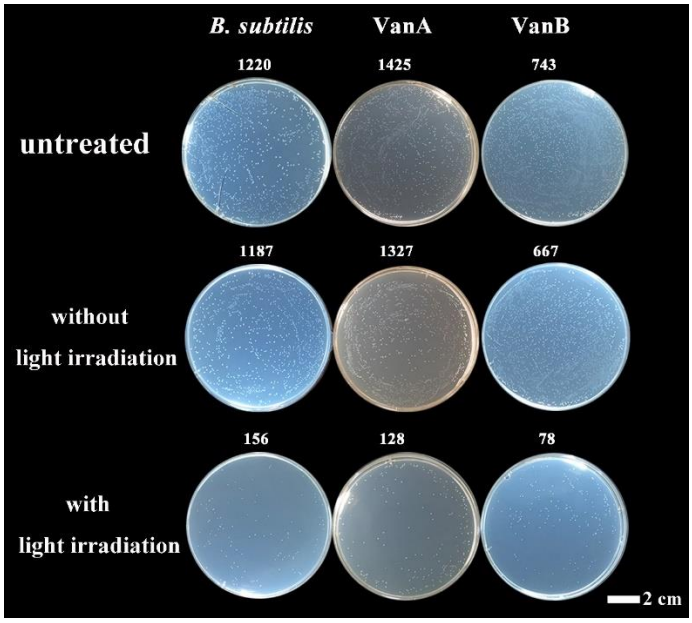

Fig. S12 Bacterial colonies of *B. subtilis* (first column), VanA (second column) and VanB (third column) on agar plates for untreated bacteria (first row), and bacteria exposed to Van-dHTz without (second row) and with (third row) 808 nm light irradiation (5 min). The concentration of Van-dHTz used are 0.09, 27.1 and 9.6 μg mL^{-1} for *B. subtlis*, VanA and VanB, respectively. The CFU values are presented above each plate.

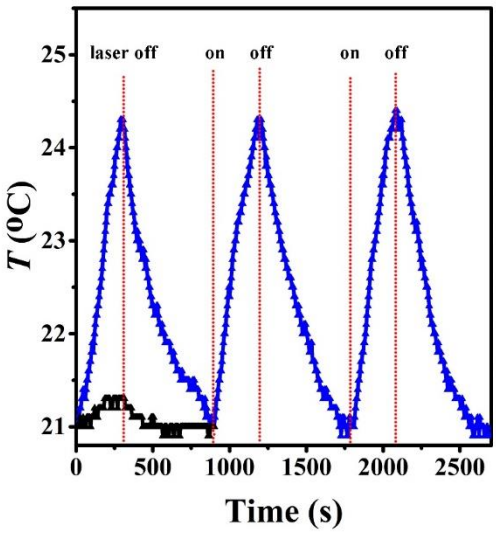

Fig. S13 Temperature (*T*) change profile of cit-capped UCNC suspended in PBS buffer (25 mg in 1 mL) excited using 808 nm light (4.3 W cm^{-2}) (blue). The *T vs*. time profiles for three laser 'on'/'off' cycles are shown. Temperature profile of PBS buffer irradiated with the same light source for 5 min (black). In this case, the temperature increases only by 0.3 °C.

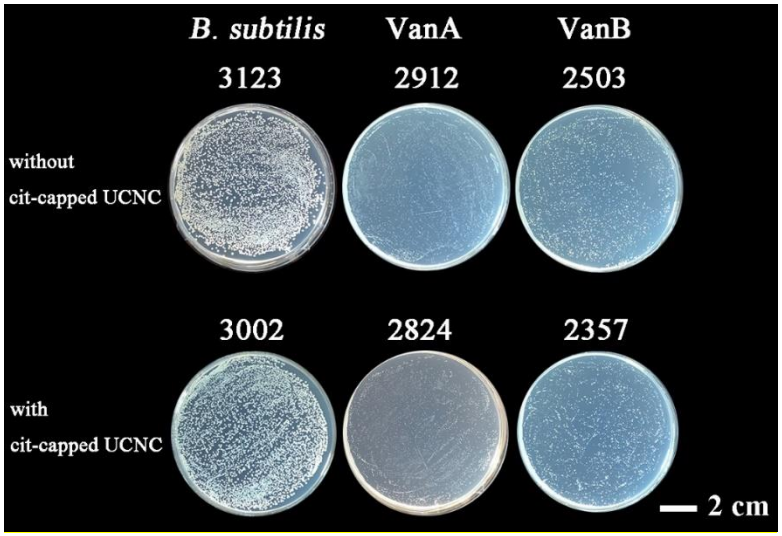

Fig. S14 Colonies of *B. subtilis* (first column), VanA (second column), and VanB (third column) cells on agar plates for bacteria exposed to 808 nm light for 5 min (top row), and bacteria exposed to 808 nm light for 5 min in the presence of cit-capped UCNC (bottom row). The CFU values are presented above each plate.

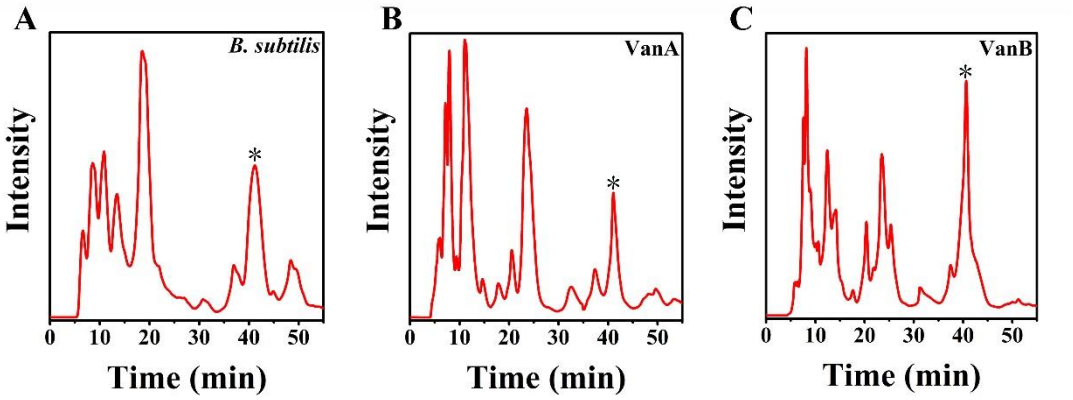

Fig. S15 HPLC separation of UDPMurNAC-pp from *B. subtilis* (A), VanA (B), VanB (C) monitored at 260 nm. The Nb-functionalized bacteria are mixed with TB-UCNC and irradiated with 808 nm light for 5 min. The peak eluted at ~ 40 min (*) corresponds to UDPMurNAC-pp identified by LCMS.

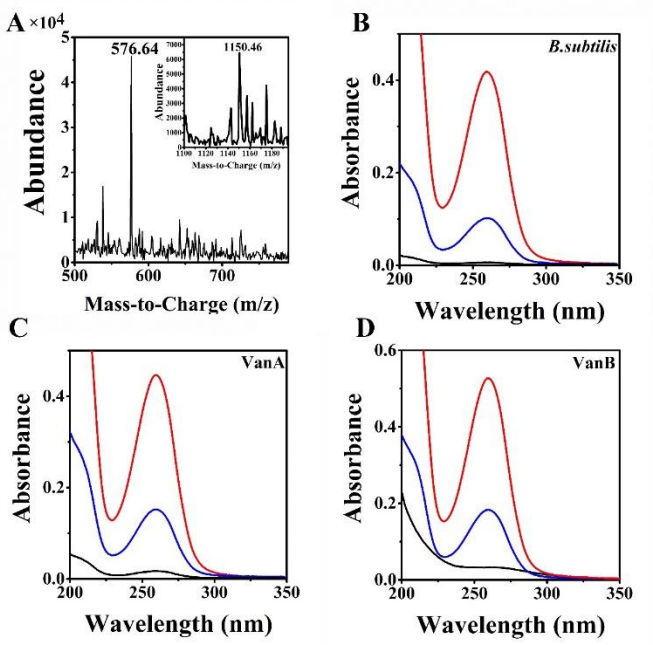

Fig. S16 (A) Identification of UDPMurNAc-pp, separated using HPLC, by LCMS. The absorption spectra of UDPMurNAc-pp with λ_{max} = 260 nm for *B. subtilis* (B), VanA (C) and VanB (D). The concentrations of Van and Van-dHTz (in terms of Van concentration) used are 0.11, 27.0 and 8.9 μg mL^{-1} for *B. subtilis*, VanA and VanB, respectively. Untreated bacteria (black) show the least amount of UDPMurNAc-pp followed by bacteria treated with Van (blue). The highest amount of UDPMurNAc-pp is observed for bacteria with bound Van after NIR light activation in the presence of TB-UCNC (red).

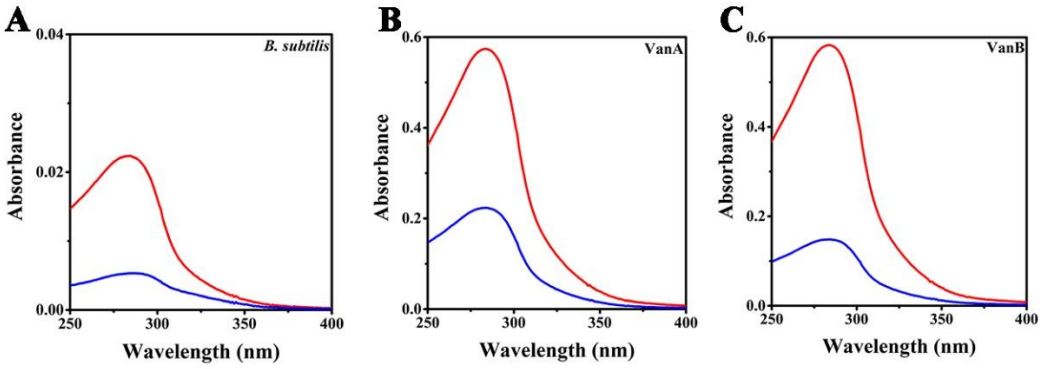

Fig. S17 Absorption spectra of unbound Van-dHTz before (red) and after (blue) 808 nm light exposure for *B. subtils* (A), VanA (B) and VanB (C). Concentrations of unbound Van before light exposure were 0.093, 17.9 and 6.1 μM for *B. subtilis*, VanA and VanB, respectively. Concentrations of unbound Van after light exposure were 0.022, 7.0 and 1.5 μM for *B. subtilis*, VanA and VanB, respectively.

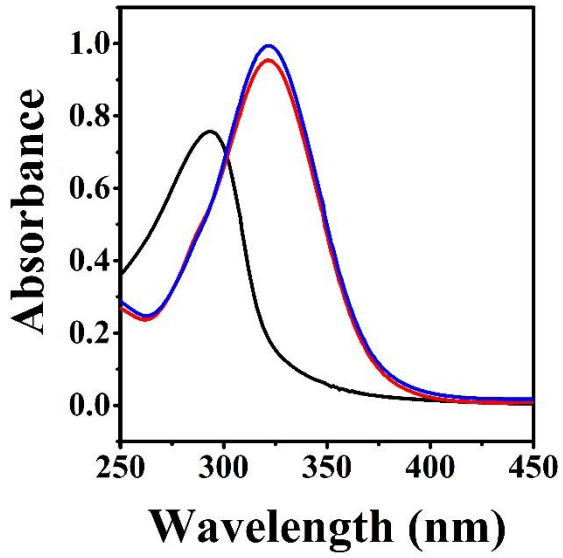

Fig. S18 UV-vis absorption of dHTz **1a** (black), and **1a** after 808 nm light (4.3 W cm^{-2}) exposure for 5 min in the presence of TB-UCNC (25.0 mg mL^{-1}) with (red) and without (blue) a thin slice of chicken breast meat placed between the reactor and light source.

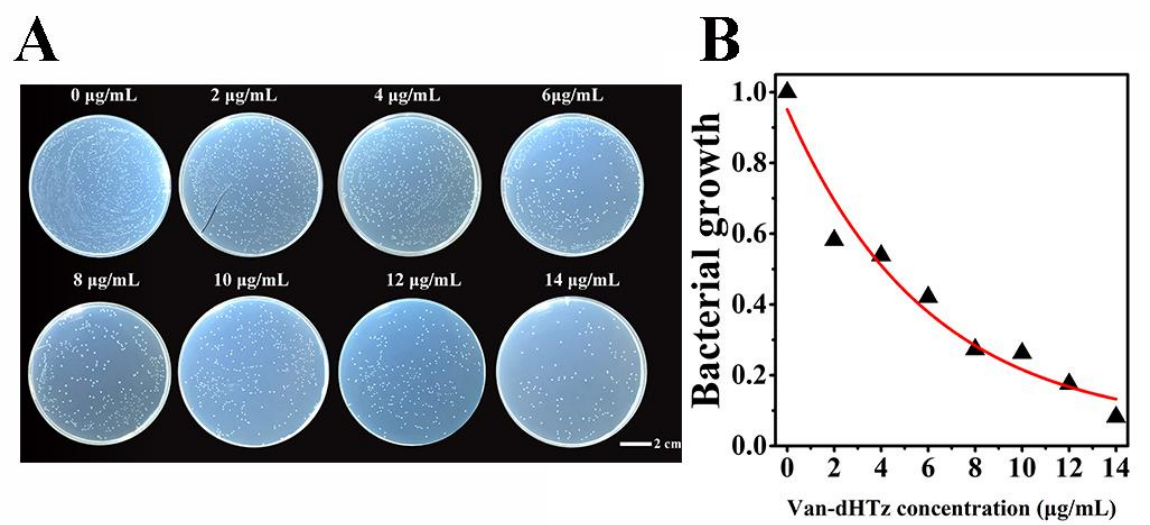

Fig. S19 (A) VanB bacterial colonies on agar plate after treatment with various concentrations of Van-dHTz in the presence of TB-UCNC (25 mg mL^{-1}) and 808 nm light irradiation through a 1.5 mm thick chicken breast for 5 min. (B) Relative bacterial growth after treatment with various concentrations of Van-dHTz with respect to growth in the absence of Van-dHTz. The data points were fit to a single-exponential decay function, and the MIC value taken to be the minimum concentrations of Van needed to give 90% growth inhibition.

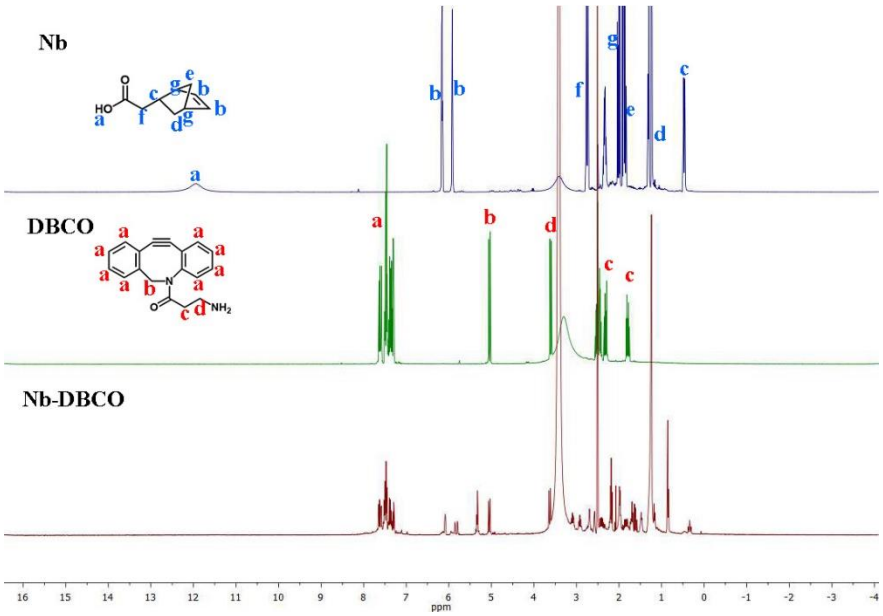

Fig. S20 ^{1}H NMR spectra of Nb, DBCO and Nb-DBCO. Peak assignments for Nb and DBCO based on ref. R5 and R6.

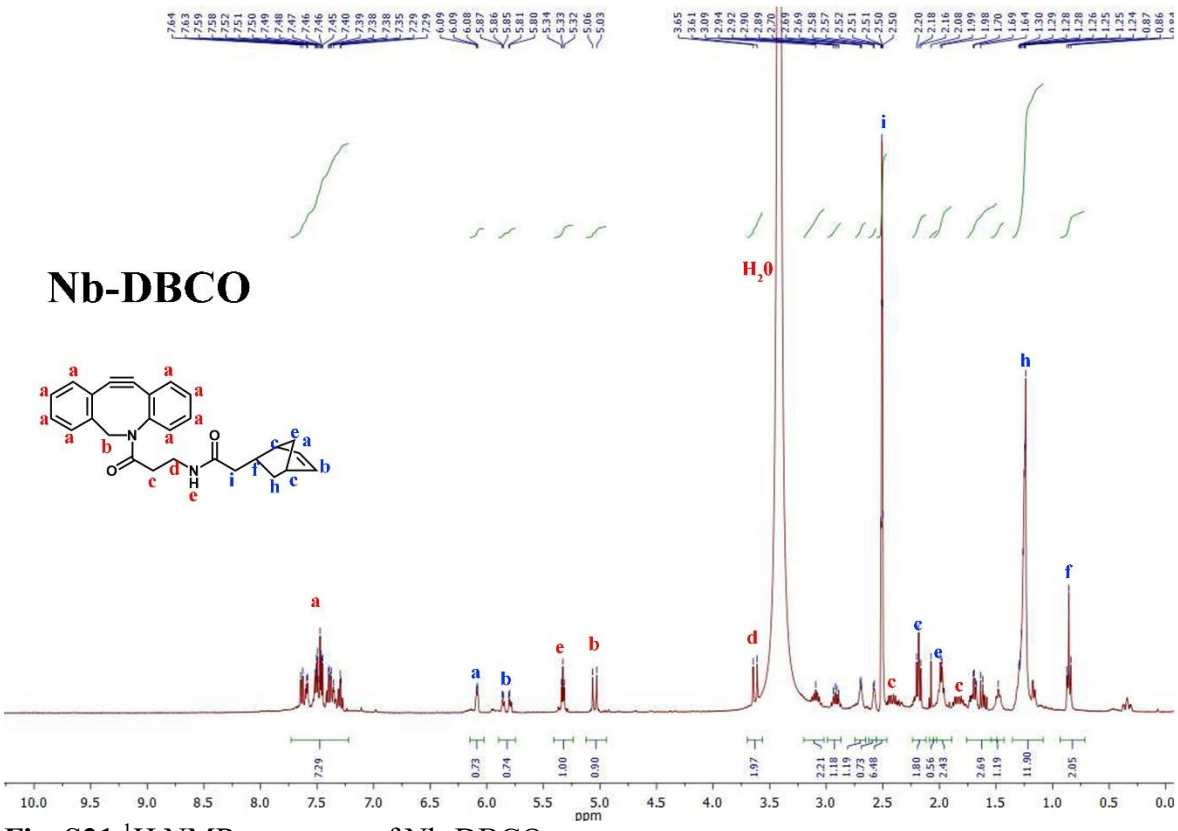

Fig. S21 ^{1}H NMR spectrum of Nb-DBCO.

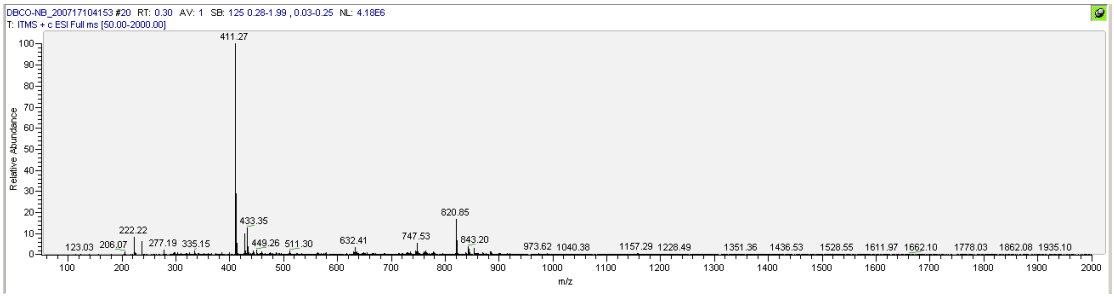

Fig. S22 LCMS spectrum of Nb-DBCO.

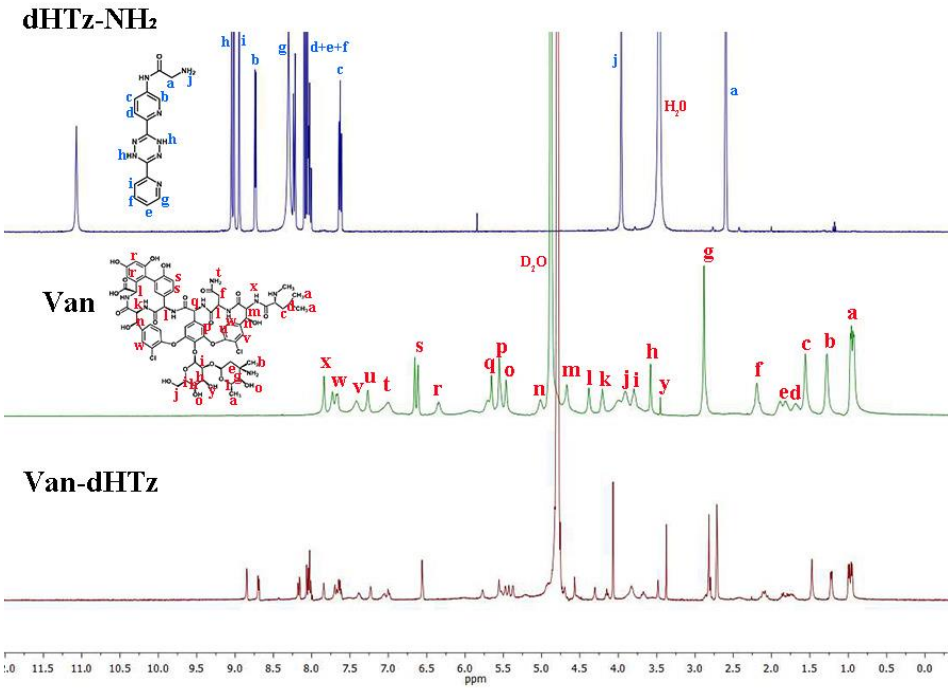

Fig. S23 ^{1}H NMR spectra of dHTz-NH_2, Van and Van-dHTz. Peak assignments for dHTz-NH_2 and Van based on ref. R7 and R8.

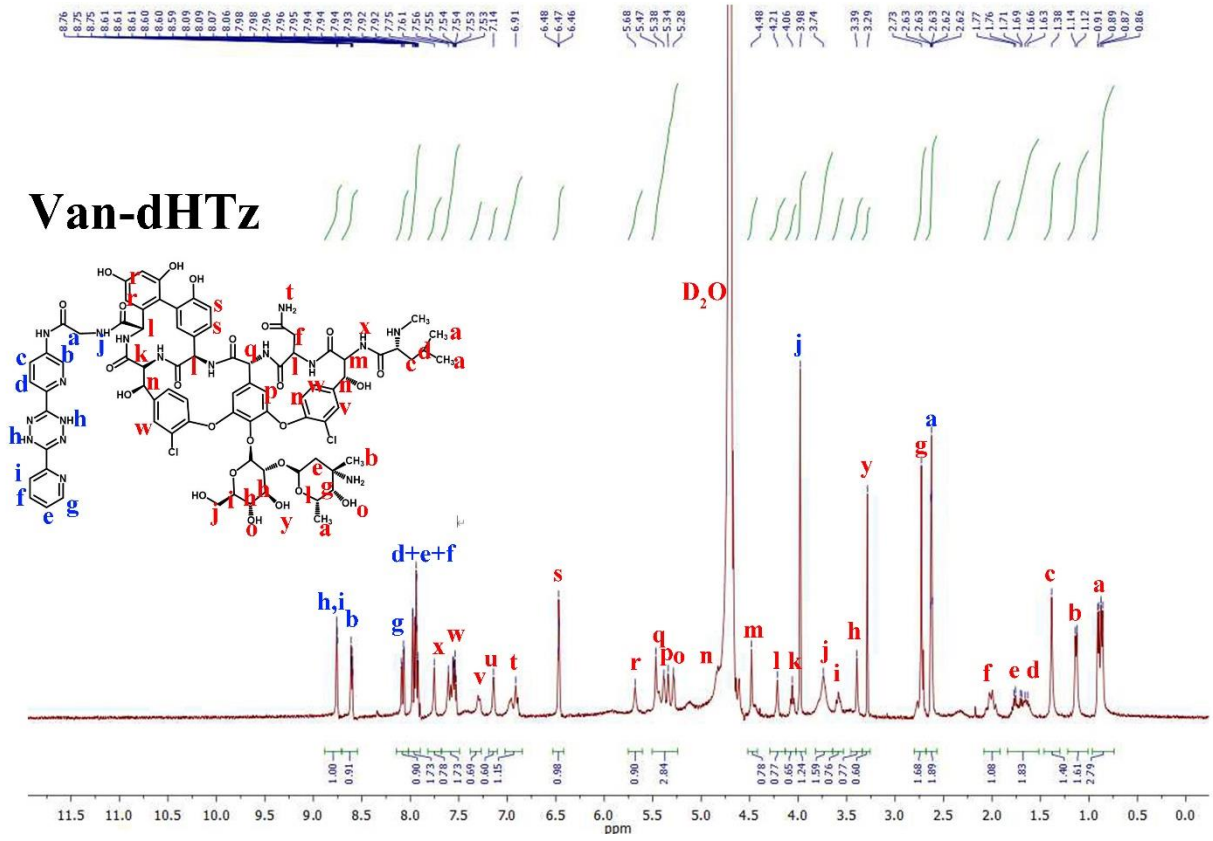

Fig. S24 ^{1}H NMR spectrum of Van-dHTz.

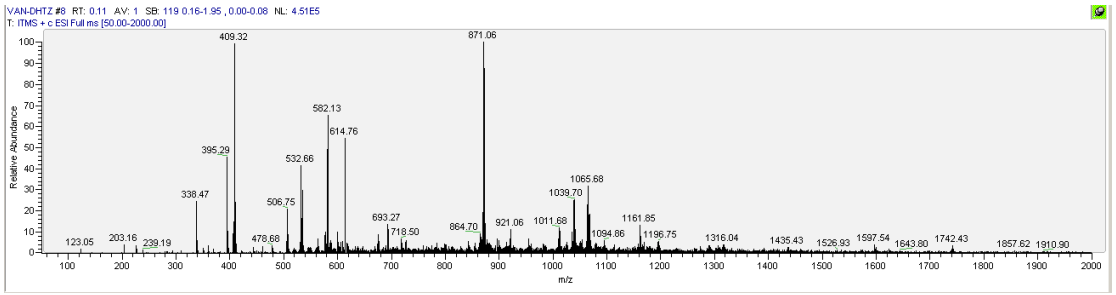

Fig. S25 LCMS spectrum of Van-dHTz.

References

R1. B. Xue, D. Wang, L. Tu, D. Sun, P. Jing, Y. Chang, Y. Zhang, X. Liu, J. Zuo, J. Song, J. Qu, E. J. Meijer, H. Zhang, X. Kong, *J. Phys. Chem. Lett.*, 2018, **9**, 4625-4631.

R2. L. E. Mackenzie, J. A. Goode, A Vakurov, P. P. Nampi, S. Saha, G. Jose, P. A. Millner, *Sci. Rep*., 2018, **8**, 1106.

R3. Y. Zhong, G. Tian, Z. Gu, Y. Yang, L. Gu, Y. Zhao, Y. Ma, J. Yao, *Adv. Mater*., 2014, **26**, 2831-2837.

R4. J. Gil-Tomás, S. Tubby, I. P. Parkin, N. Narband, L. Dekker, S. P. Nair, M. Wilson, C. Street, *J. Mater. Chem*., 2007, **17**, 3739-3746.

R5. A. J. Gormley, J. Yeow, G. Ng, O. Conway, C. Boyer, R. Chapmen, *Angew. Chem. Int. Ed*., 2018, **57**, 1557-1562.

R6. O. Sannikov, S. Hanson, P. Saunders, K. L. Branch, N. Merbouh, *J. Lab. Chem. Educ*., 2019, **7**, 8-18.

R7. V. X. Truong, K. M. Tsang, F. Ercole, J. S. Forsythe, *Chem. Mater*., 2017, **29**, 3678-3685.

R8. C. M. Pearce, D. H. Williams, *J. Chem. Soc. Perkin Trans*. 2, 1995, 153-157.