Supplementary Information

Unraveling site-specific dopant behavior governing antibacterial activity in doped titanate nanosheets

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Detailed explanation of antibacterial activity assays

(1) Turbidity method

Bacterial growth kinetics were monitored by measuring the turbidity (equal to the optical density). A standardized bacterial inoculum (typically 1×10^7 CFU/mL) was inoculated into TSB broth containing *x*M-TNS samples at the specified concentrations in a 96-well transparent microplate with a flat bottom. The microplate was incubated at 37°C for a maximum of 24 h with gentle shaking. During incubation, the turbidity of each well was recorded at $\lambda = 600$ nm at regular time intervals using a microplate reader to monitor bacterial growth. To minimize variability in the dataset, four wells containing identical bacterial suspensions were prepared for each experimental condition, and the mean turbidity of these wells was used for subsequent data analysis.

(2) Colony Counting Method

To quantify the bactericidal effect, aliquots of the bacterial culture (initial concentration: 1×10^7 CFU/mL) treated with 20 mM or 5 mM *x*M-TNS samples were collected after incubation for 24 h at 37ºC. The samples were serially diluted with sterile 0.9% NaCl solution, and a constant solution volume with various dilution factors was plated on an TSB agar plate in a petri dish. The plates were incubated at 37℃ for 24 h, and then the number of colonies was counted to determine the viable cell concentration (CFU/mL). Colony counting assays were performed in triplicate, and the data were expressed as the average of three independent samples.

(3) Disk Diffusion Method

The disk diffusion method is generally used to evaluate diffusion-mediated mechanisms. Sterile filter paper disks (diameter: 6 mm) were impregnated with 20 μL of 200 or 500 mM TNS, *x*Cu-TNS, or 3.6Ag-TNS colloidal solution and air-dried. These disks were placed onto TSB agar plates after spreading the standardized bacterial suspension (0.5 McFarland standard: approximately 1.5×10^8 CFU/mL) as uniformly as possible using a platinum inoculating loop. After incubation at 37℃ for 24 h, a photograph of the agar plate was taken and used to determine the diameter of the inhibition zones.

(4) Disk Diffusion Method WST-1 Membrane Metabolic Activity Assay

WST-1 Membrane Metabolic Activity Assay

To evaluate the effect of *x*Cu-TNS on the metabolic activity of bacteria, a WST-1 reduction assay was performed. Bacterial suspensions (approximately 1.5×10^8 CFU/mL) were incubated with the nanosheet samples at 37 °C. At designated time intervals, the WST-1 reagent was added to the aliquots according to the manufacturer's protocol, followed by further incubation for 4 hours in the dark. The metabolic activity and bacterial morphological integrity were simultaneously monitored by measuring the absorbance of the produced formazan dye at 450 nm and the optical density at 600 nm, respectively, using a microplate reader

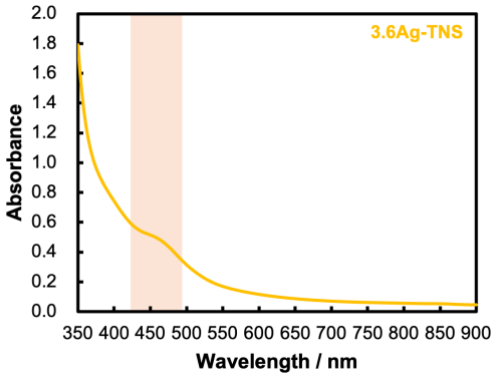

Fig. 1S Photoabsorption spectrum of colloidal solution of 5 mM 3.6Ag-TNS.

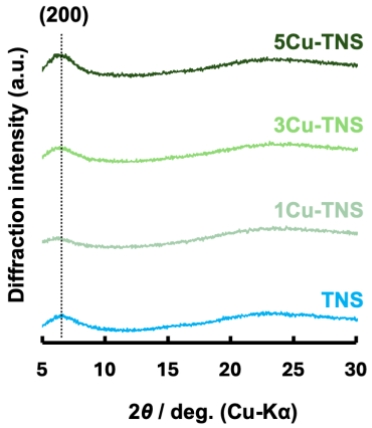

Fig. 1S XRD patterns of undoped TNS and xCu-TNS, where thin film samples for measurement were fabricated by drying colloidal solutions on a glass plate. The peak attributed to the (200) plane of tetratitanate is indicated by the dotted line.

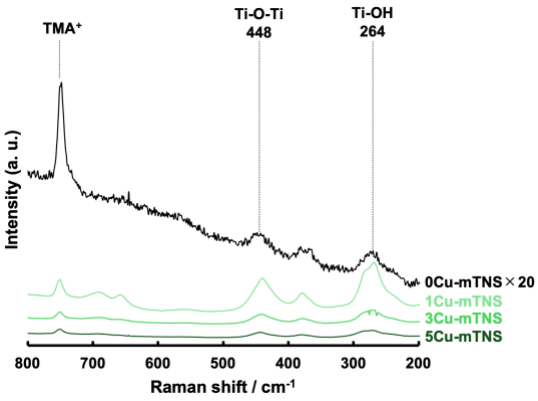

Fig. 3S Raman spectra of TNS and *x*Cu-TNS, where the measurements were undertaken for the identical samples to Fig. 2S.

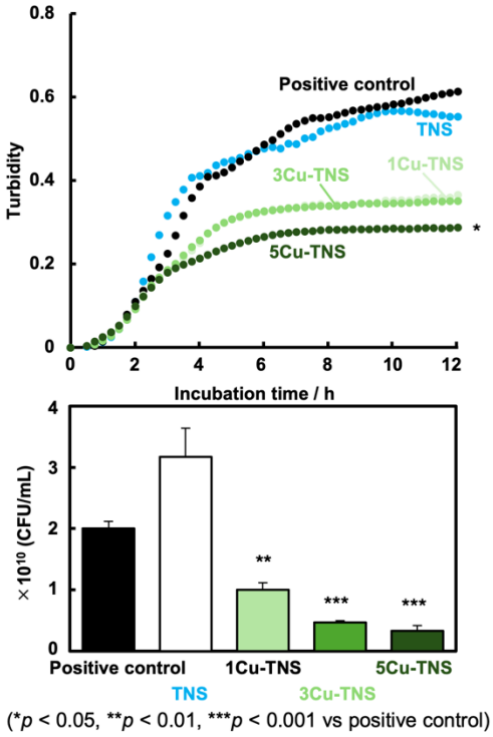

Fig. 4S (a) Changes in the turbidity of *E. coli* suspensions (1×10^7 CFU/mL) in the absence or presence of 20 mM *x*Cu-TNS with several Cu doping levels. (b) The antibacterial activity of *x*Cu-TNS against *E. coli* was determined using the colony counting method. The aliquots of the bacterial suspensions in (a) after 24 h incubation were utilized for the tests, and the viability of bacterial cells is shown as colony forming units per unit volume of the aliquot (CFU/mL).

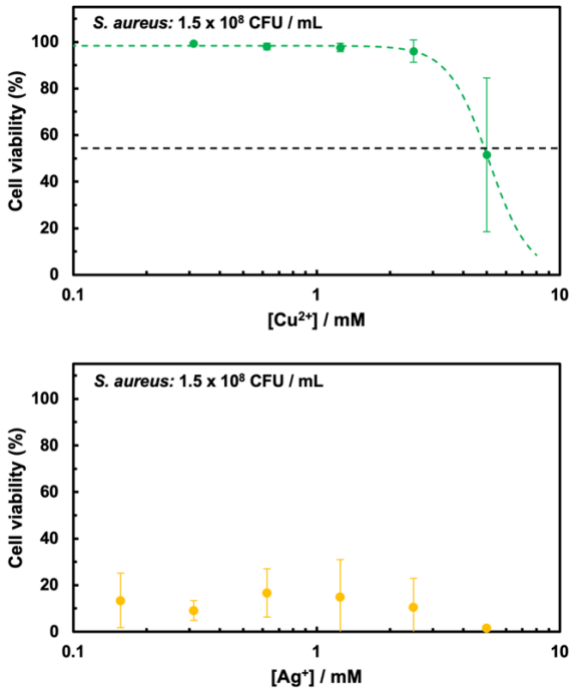

Fig. 5S Minimum inhibitory concentration (MIC) of the metal salt precursors, $CuCl_2$ and $AgNO_3$, against *S. aureus* (1.5×10^8 CFU/mL). Bacterial viability (%) was determined relative to untreated control. $AgNO_3$ exhibited potent antibacterial activity even at the lowest concentration tested (0.156 mM), confirming that free Ag^+ ions are intrinsically active across the concentration range relevant to Ag-TNS. In contrast, $CuCl_2$ showed no significant reduction in viability up to 2.5 mM, with the MIC occurring at approximately 5 mM, a concentration substantially exceeding any amount of Cu ions that could feasibly be released from 3.9Cu-TNS, thereby excluding ion leaching as the primary mechanism of antibacterial activity in Cu-TNS.

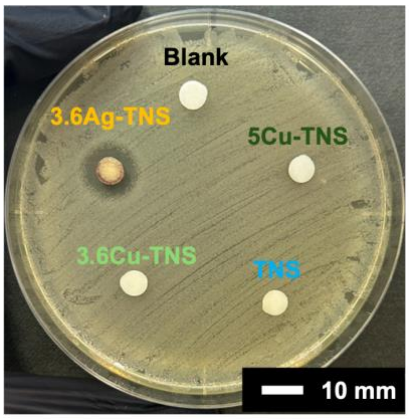

Fig. 6S Inhibition zone toward *E. coli* surrounding the paper filter disks impregnated with 10 μmol of TNS, *x*Cu-TNS, or 3.6Ag-TNS.

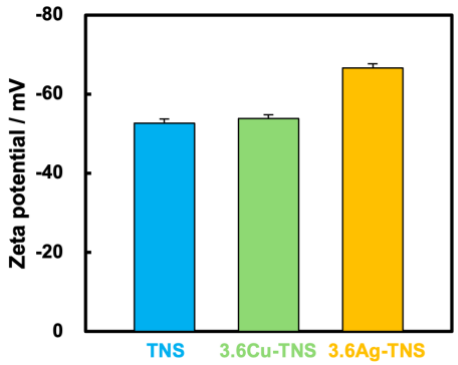

Fig. 7S Zeta potentials of colloidal solutions of TNS, 3.6Cu-TNS, and 3.6Ag-TNS dispersed in 0.1 M tris-HCl at pH 7.4.

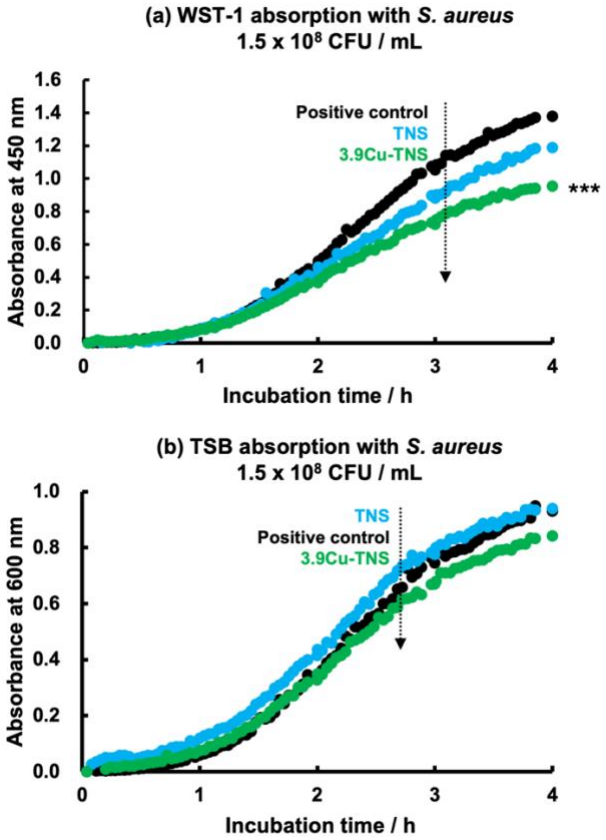

Fig. 8S WST-1 membrane-bound metabolic activity assay of *S. aureus* (1.5×10^8 CFU/mL) in the presence of positive control (bacterial suspension only), TNS and 3.9Cu-TNS. (a) Time-step of formazan absorbance at 450 nm, reflecting membrane-associated respiratory activity. The 3.9Cu-TNS group exhibited markedly suppressed formazan production relative to the positive control (***$p < 0.001$ at 4 h). (b) Simultaneously recorded turbidity at 600 nm. No significant difference was observed among the three groups, indicating that metabolic impairment at the cell surface preceded any detectable change in bacterial density.