## **Supplementary Information**

# Rapid naked-eye detection of Gram-positive bacteria by vancomycin-based nano-aggregation

Cheong Shin<sup>‡a</sup>, Ha Neul Lee<sup>‡a</sup>, Jea Sung Ryu<sup>a</sup>, Hyun Jung Chung<sup>\*a,b</sup>

<sup>a</sup>Graduate School of Nanoscience and Technology, <sup>b</sup>Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Daejeon 305-701, South Korea

\*Corresponding author: <u>hyunjc@kaist.ac.kr</u> \*Authors contributed equally

#### **Supplementary methods**

#### NB-vanco assay in serum

Human serum was obtained by gradient centrifugation of human whole blood (Innovative Research, USA) at 400 g for 10 min, and then at 1500 g for 20 min. Cultured *S. aureus* or *E. coli* (1.25 x 10<sup>7</sup>) were then added into 100 ul of human serum or bovine serum (WELGENE, Korea), and centrifugation was performed at 300 g for 5 min. The bacterial pellet was re-suspended in 100  $\mu$ l of PBS++, and 1.59 x 10<sup>9</sup> of NB-vanco or NB were added. After 30 min, the absorbance of the upper part of the assay solution was measured using Nanophotometer P330 (IMPLEN, Germany) or the whole assay solution was applied through the porous filter system and images of the filter membranes were captured using iPad.

### Supplementary figures



Fig. S1. Scanning electron microscopy of NB (left) and NB-vanco (right).



**Fig. S2.** Quantification of aggregation signals by image analysis of the NB-vanco assay results integrated with the porous filter system. Different b\* coordinate values in the images were counted to determine the cut-off value for positive signals. Data are presented as the mean  $\pm$  s.d. (n=2).



**Fig. S3.** Sample tube analysis from the NB-vanco assay with different types of bacteria. (A) Absorbance measurements of the assay solutions according to various assay times  $(5.0 \times 10^5 \text{ bacterial cells/µl}; ***, p < 0.001)$ . (B) Images of the sample tubes after the assay at 30 min (top) and 90 min (bottom).



**Fig. S4.** NB-vanco assay performed with bacteria spiked into serum. (A) Absorbance measurements of sample tubes (n=3; \*, p < 0.001) and (B) images of filter membrane after applying the porous filter system.