

Supporting Information

A quorum-based fluorescent probe for imaging pathogenic bacteria

Sajal Shrivastava^{a,1}, Rekha Arya^{b,1}, Kyeong Kyu Kim^{b,c,d,f}, Nae-Eung Lee^{a,c,d,e,f,*}

^aSchool of Advanced Materials Science & Engineering, Sungkyunkwan University, Suwon, Gyeonggi-do 16419, Korea

^bDepartment of Molecular Cell Biology, Sungkyunkwan University School of Medicine, Samsung Medical Centre, Suwon, 16419, Korea

^cSamsung Advanced Institute for Health Sciences & Technology (SAIHST), Sungkyunkwan University, Suwon, Gyeonggi-do 16419, Korea

^dSKKU Advanced Institute of Nanotechnology (SAINT), Sungkyunkwan University, Suwon, Gyeonggi-do 16419, Korea

^eInstitute of Quantum Biophysics (IQB), Sungkyunkwan University, Suwon, Gyeonggi-do 16419, Korea

^fBiomedical Institute for Convergence at SKKU (BICS), Sungkyunkwan University, Suwon, Gyeonggi-do 16419, Korea

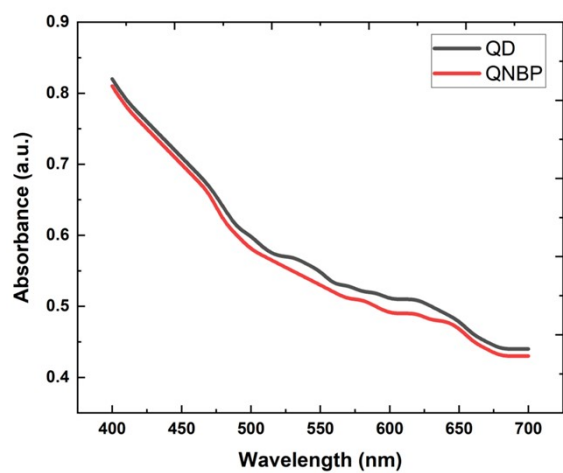


Fig S1. UV-visible spectra of bare QDs and QNBPs. The absorbance of bare QDs and post bioconjugation was measured between 400-700 nm.

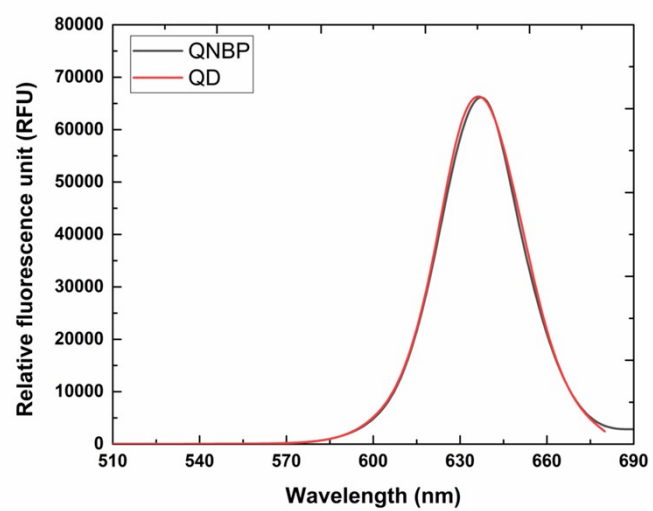


Fig S2. Fluorescence spectra of bare QDs and QNBPs.

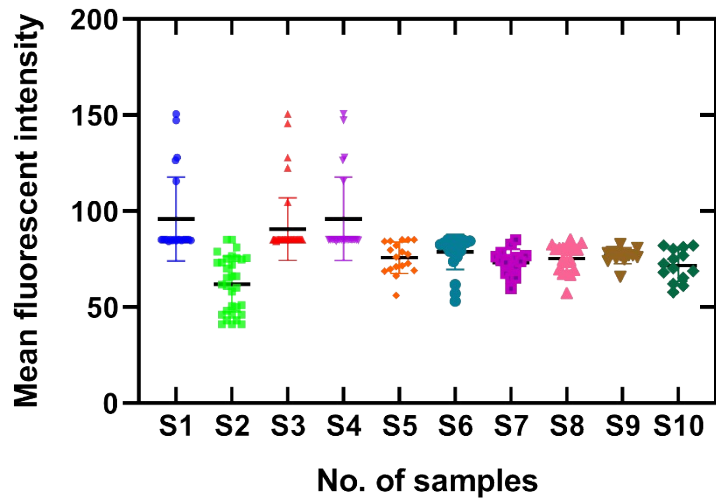


Fig. S3. Binding between QNBPs and various clinical *S. aureus* isolates. The clinical *S. aureus* isolates (S1–S10) were incubated with QNBPs, and imaging was performed. The fluorescent intensity of individual cells from all the isolates was quantified. All the strains were positive for binding with QNBPs with a similar mean fluorescence intensity.

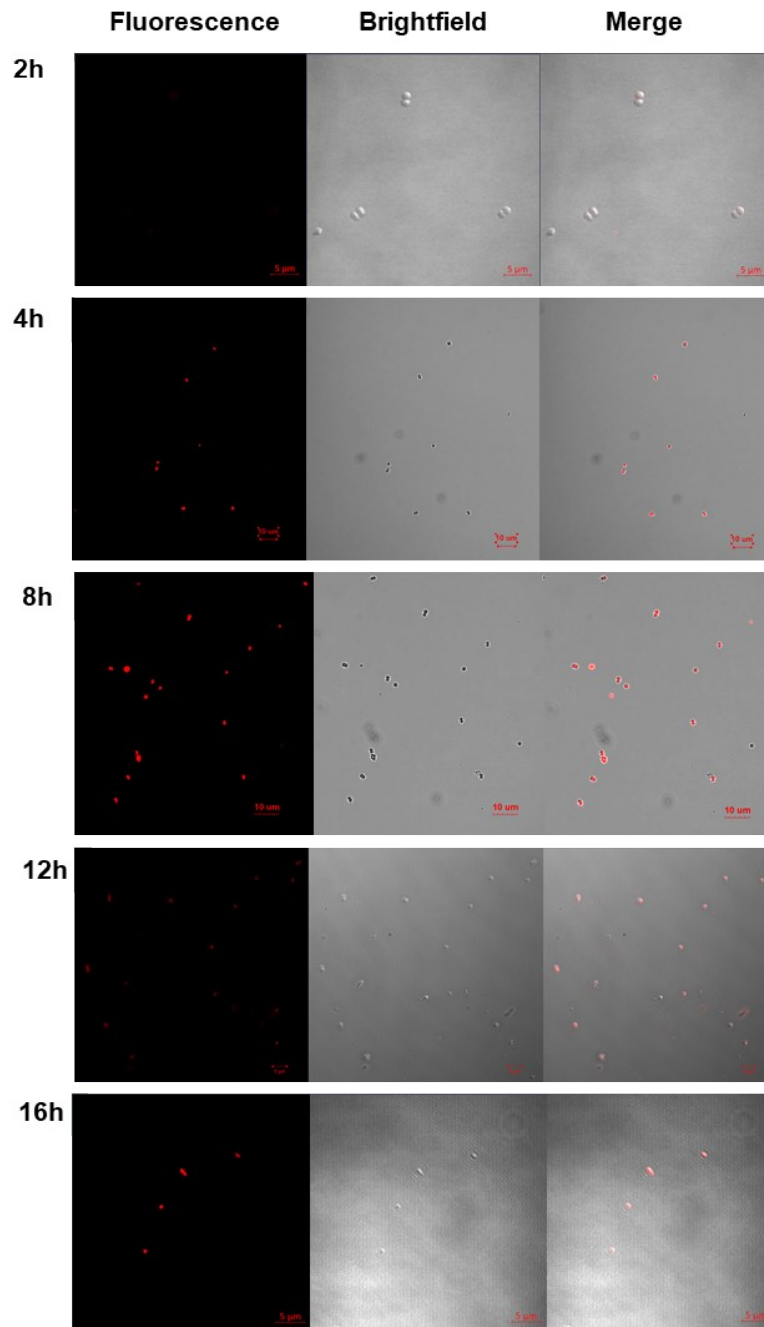


Fig. S4. Time-dependent binding of QNBPs with *S. aureus* cells. The *S. aureus* culture was collected at different time intervals (2, 4, 8, 12 and 16 h) and incubated with QNBPs, followed by imaging with a confocal microscope.

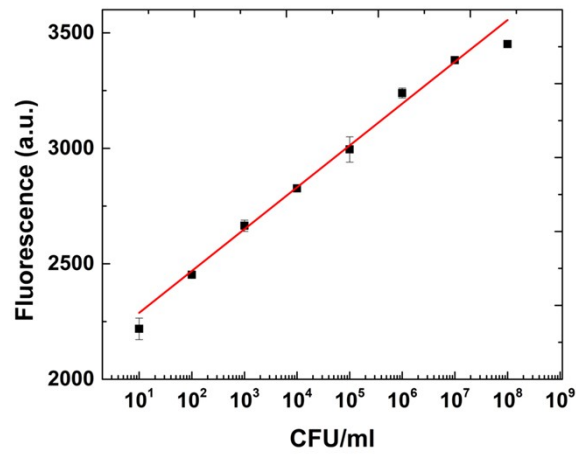


Fig. S5. The fluorescent intensity in relation to the *S. aureus* bacterial count. *S. aureus* cultures with different cell counts were incubated with QNBPs, and the fluorescence intensity was measured. The analysis revealed that the coefficient of determination (R^2) was 0.988. The error bar represents \pm SEM.

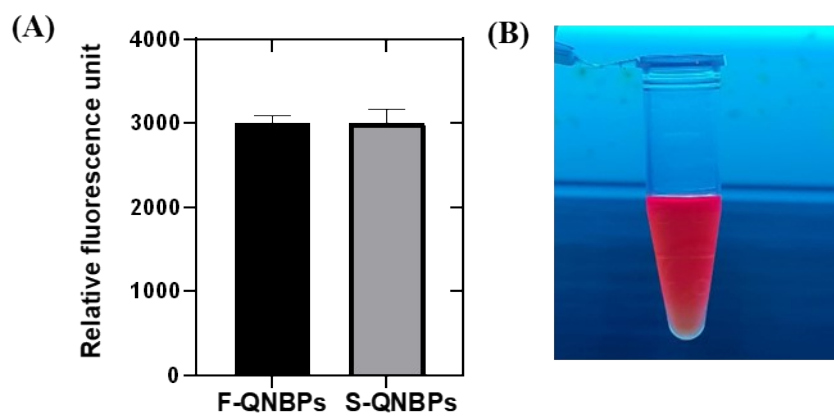


Fig. S6. The stability of QNBPs. (A) The binding comparison of freshly prepared QNBPs (F-QNBPs) and stored QNBPs (S-QNBPs). (B) The QNBPs solution was stored for up to a month under UV light. No visible aggregation was observed.

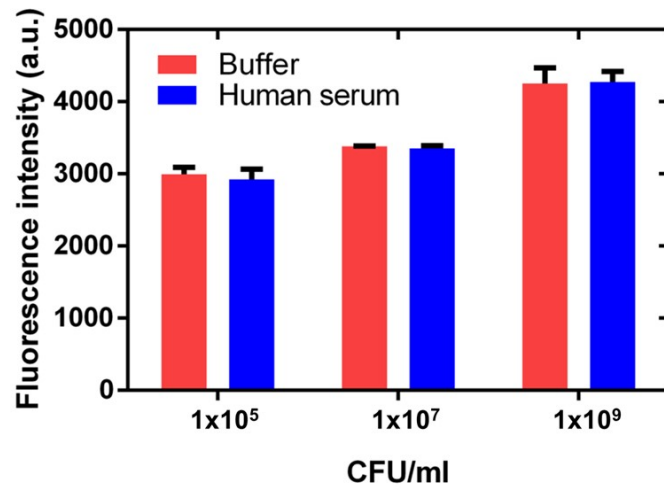


Fig. S7. The comparison between buffer solution and human serum for *S. aureus* labeling and fluorescence-based quantification. The *S. aureus* cells at multiple concentrations were used to test the binding of QNBPs in two different media.

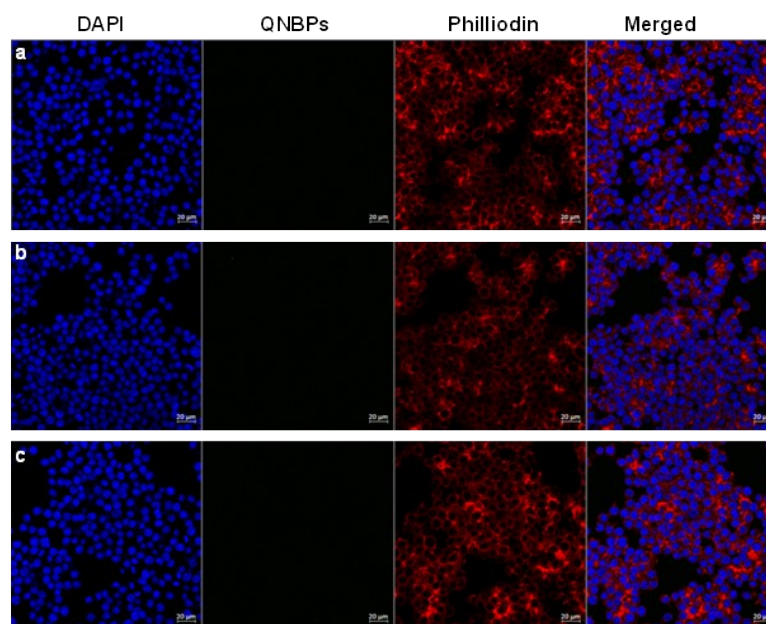


Fig. S8. Analysis of QNBP uptake by RAW264.7 cells. **(A)** RAW264.7 cells grown without QNBPs as the control and stained with rhodamine-phalloidin and DAPI. To evaluate the interaction between immune cells and the developed probe, the macrophages were treated with either 0.04 nM or **(B)** a two-fold increased concentration of QNBPs. **(C)** Fluorescence images were acquired 12 h later. As can be seen, the tested concentrations did not interact with macrophage cells.

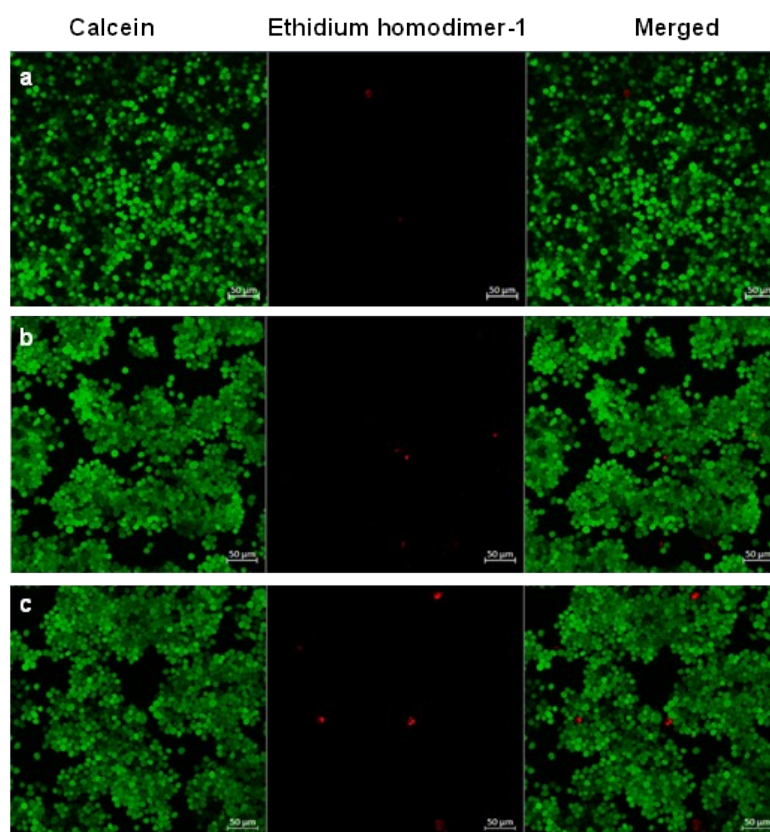


Fig. S9. The Live/Dead cell viability assay of RAW264.7 cells treated with QNBPs. The murine macrophage cells were incubated with calcein-AM and ethidium homodimer-1 and subsequently imaged with confocal microscopy to assess the cytotoxic effects of the QNBPs. **(A)** The cell culture that did not receive QNBPs and **(B)** the culture treated with the *in vivo*-tested concentration of 0.04 nM and **(C)** a two-fold increased concentration of QNBPs.

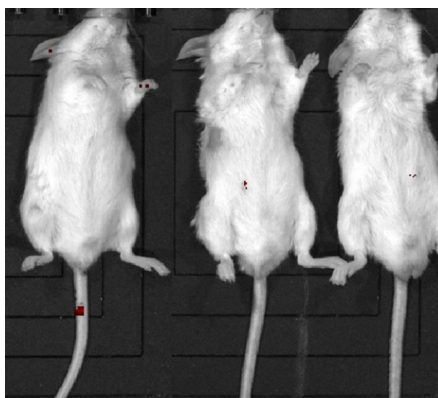


Fig. S10. The in-vivo micrographs of uninfected animals incubated with QNBPs. No significant signals from QNBPs were observed *in-vivo*.

Table 1. List of primers used in this study.

| Gene | Primers | |
|----------------|----------------|-------------------------------------|
| <i>agr-I</i> | F | 5'-CAC TTA TCA TCA AAG AGC C-3' |
| | R | 5'-CCA CTA ATT ATA GCT GG-3' |
| <i>agr-II</i> | F | 5'-GTA GAG CCG TAT TGA TTC C-3' |
| | R | 5'-GTA TTT CAT CTC TTT AAG G-3' |
| <i>agr-III</i> | F | 5'-CTG CAT TTA TTA GTG GAA TAC G-3' |
| | R | 5'-GTT TCA TTT CTT TAA GAG-3' |
| <i>agr-IV</i> | F | 5'-CAC TTA TCA TCA AAG AGC C-3' |
| | R | 5'-GTA TTT CAT CTC TTT AAG G -3' |