Supplementary information

Nanoparticle loaded hydrogel for the light-activated release and photothermal enhancement of antimicrobial peptides

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Materials and Methods

Minimum inhibitory concentration measurements

The antimicrobial properties of IK8 were tested upon the Gram-positive *S. aureus* and Gram-negative *P. aeruginosa* using the broth microdilution assay[51]. Cells were plated upon Mueller-Hinton II agar overnight. 3-5 isolated colony forming units were picked and cultivated in MH2 broth at 37°C and shaken at 300 rpm. The bacterial suspensions were then diluted in broth to achieve an optical density of 0.07 at 600 nm (OD_{600}), corresponding to 0.5 McFarland standard ≈ 1.5×10^8 CFU mL^{-1}, before a further 100-fold dilution. A serial dilution of the IK8 peptide was performed in MH2 broth before addition of 100 µL of the peptide solution to a 96-well plate

along with an equal volume of the bacterial suspension, providing final peptide concentrations of 8 – 250 μg mL^{-1}. The minimum inhibitory concentration (MIC) was determined to be the concentration of peptide at which no increase in OD_{600}, was observed after an 18 h incubation.

Results

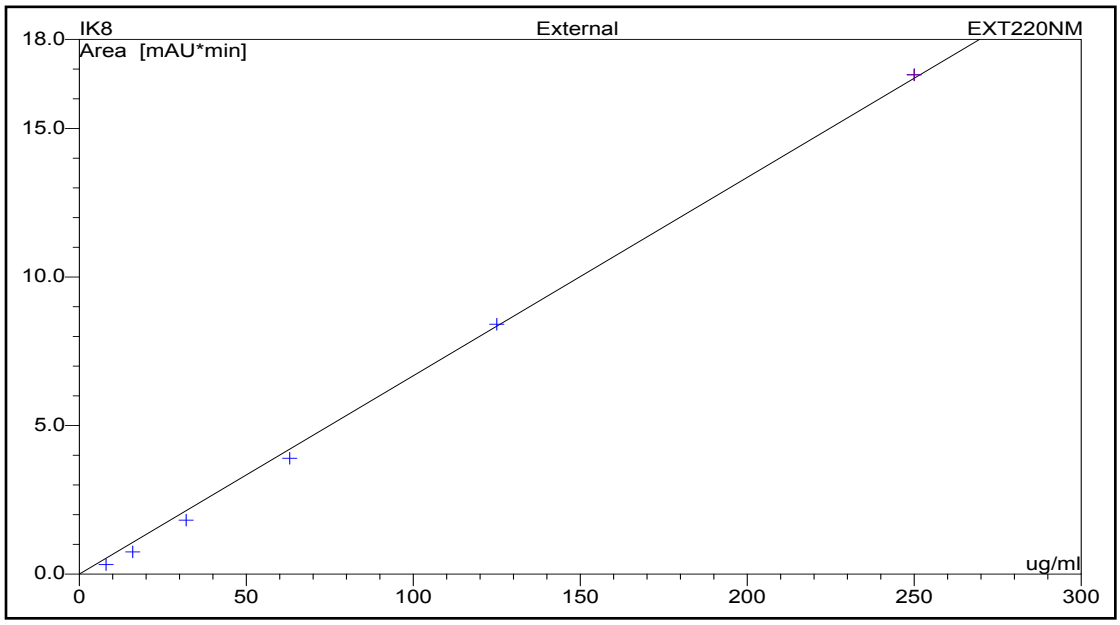

Figure S1. The absorbance at 220 nm after 5.26 min flowing increasing IK8 concentrations in an acetonitrile gradient through a 4.6 x 250 mm Insertil ODS-SP HPLC column. The IK8 concentration curve demonstrates a linear relationship between the concentration of peptide and the area beneath the 220 nm absorption peak.

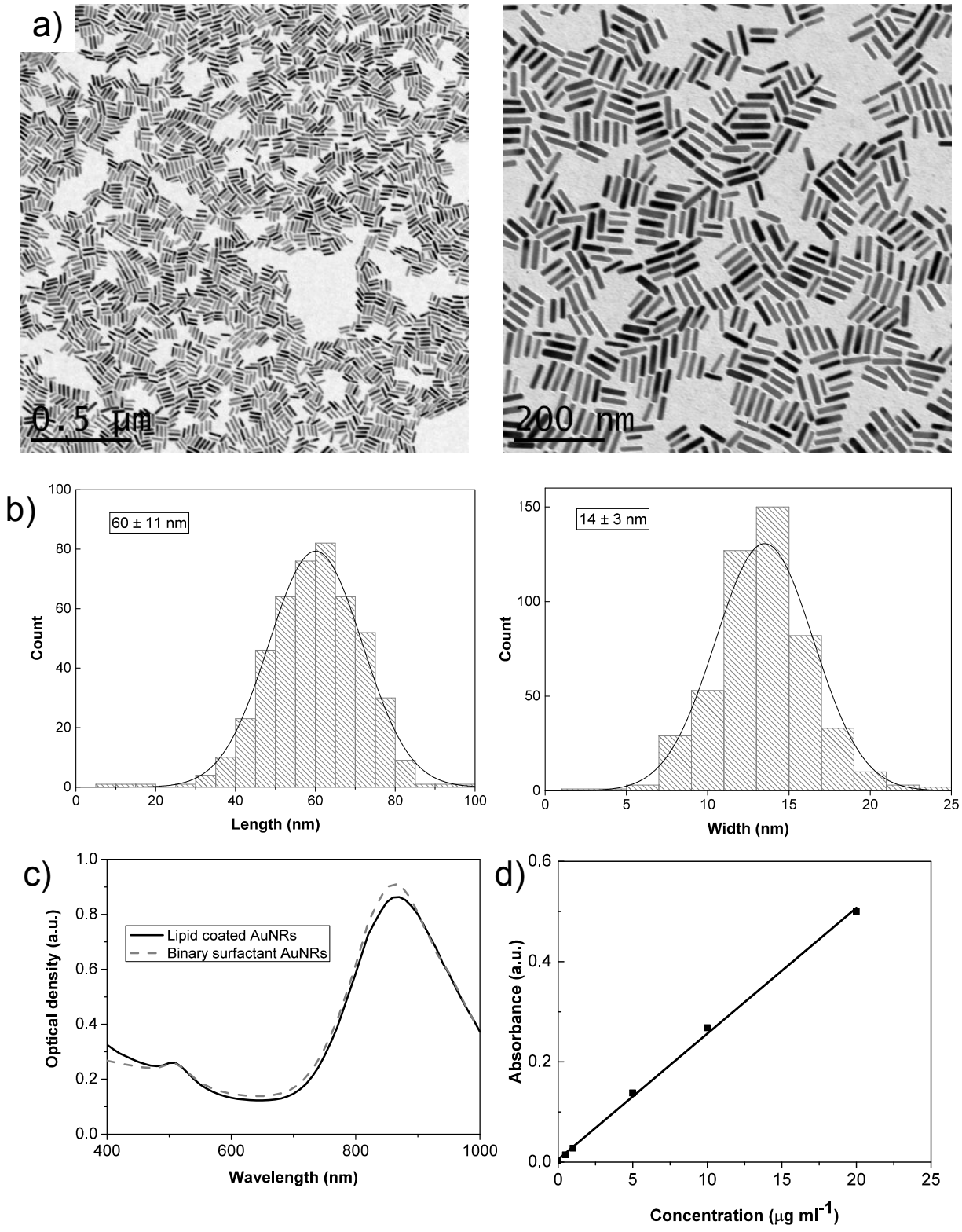

Figure S2. a) TEM images of lipid coated nanorods using a JEOL1400 at 20,000 and 25,000 times magnification (left and right images respectively. b) The length (left histogram) and width (right histogram) distributions of lipid coated AuNRs. (n= 531). c) UV-vis absorbance spectra of

binary surfactant (grey dashed line) and lipid coated (solid black line) gold nanorods. d) Atomic absorption standard curve used to determine the concentration of gold within a nanorod sample.

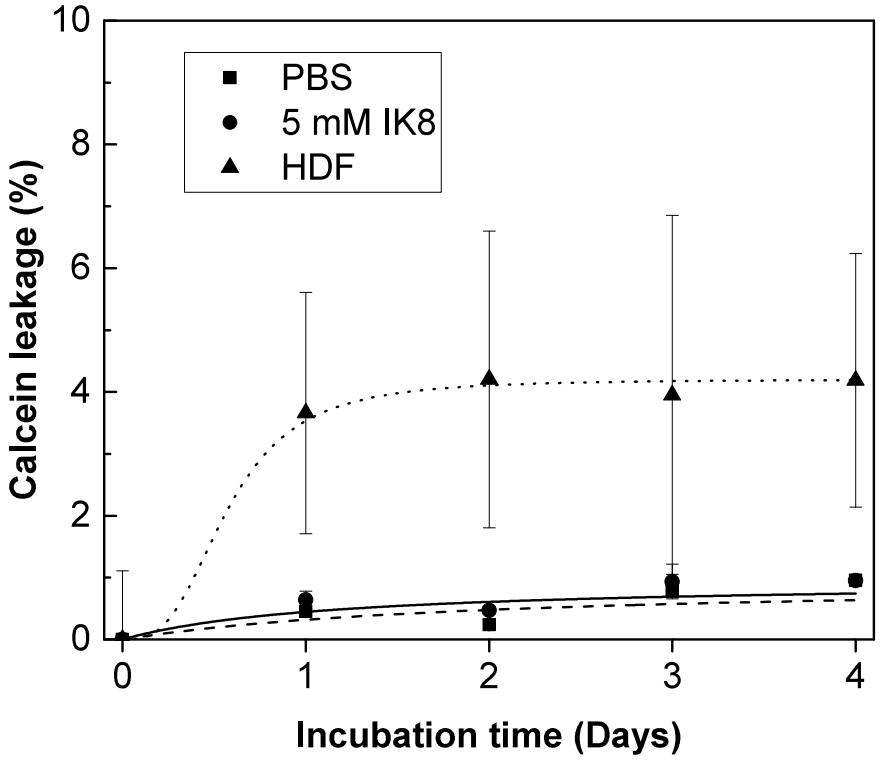

Figure S3. The calcein leakage from liposomes incubated at 37°C in PBS (solid line), 5 mM IK8 in PBS (dashed line) and HDF cells in fibroblast growth media (dotted line). Fluorescence measurements (Exc/Em 496/515 nm) were taken immediately after adding the liposomes to the well plates containing the PBS, IK8 and HDF cells, and once every 24 h for four days. 1% Triton-X100 was added to the control wells to find the maximum fluorescence, and for data normalization. Error bars indicate the standard deviation (n=6).

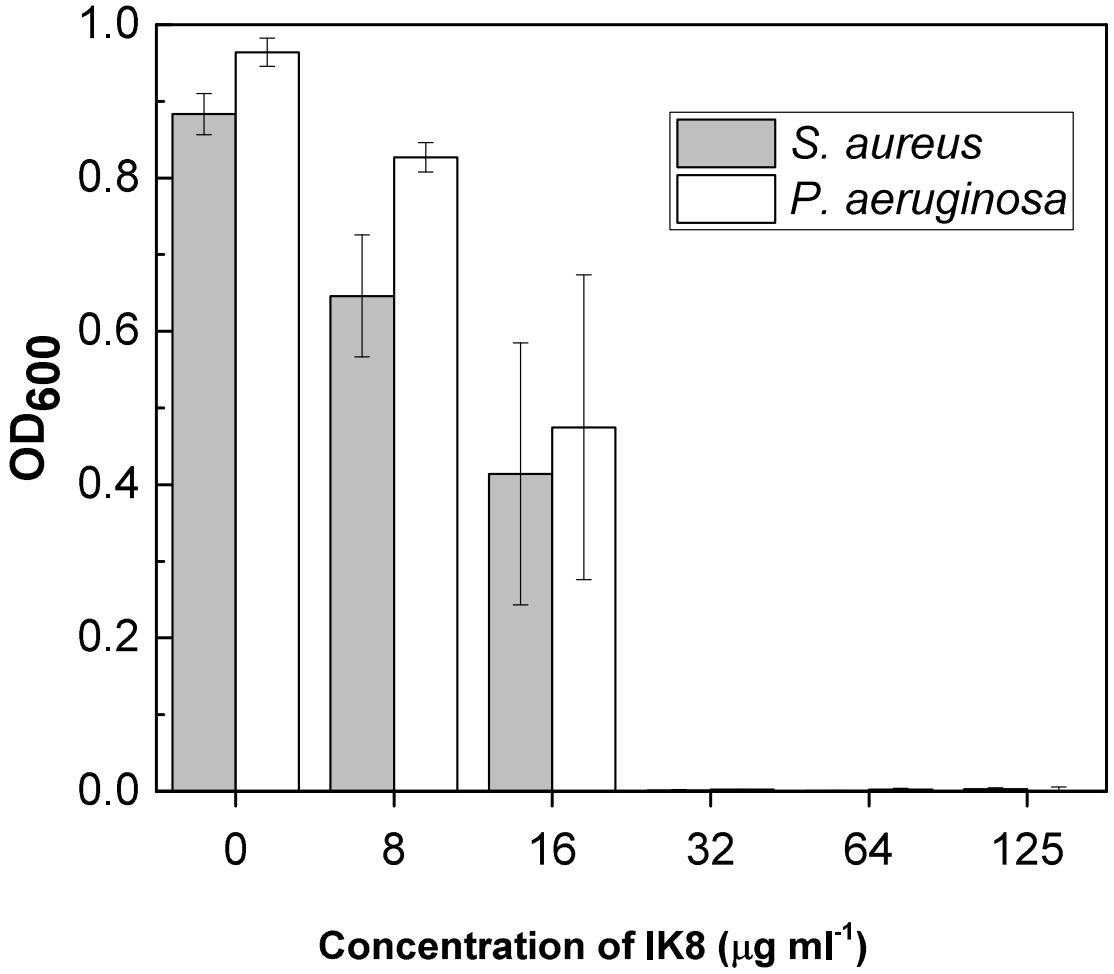

Figure S4. The change in the optical density at 600 nm of *S. aureus* and *P. aeruginosa* after an 18 h incubation with increasing concentrations of IK8 and MHB II. The minimum inhibitory concentration (MIC) is the concentration at which there is no increase in turbidity indicating no bacteria proliferation. Error bars indicate the standard deviation (n=6).

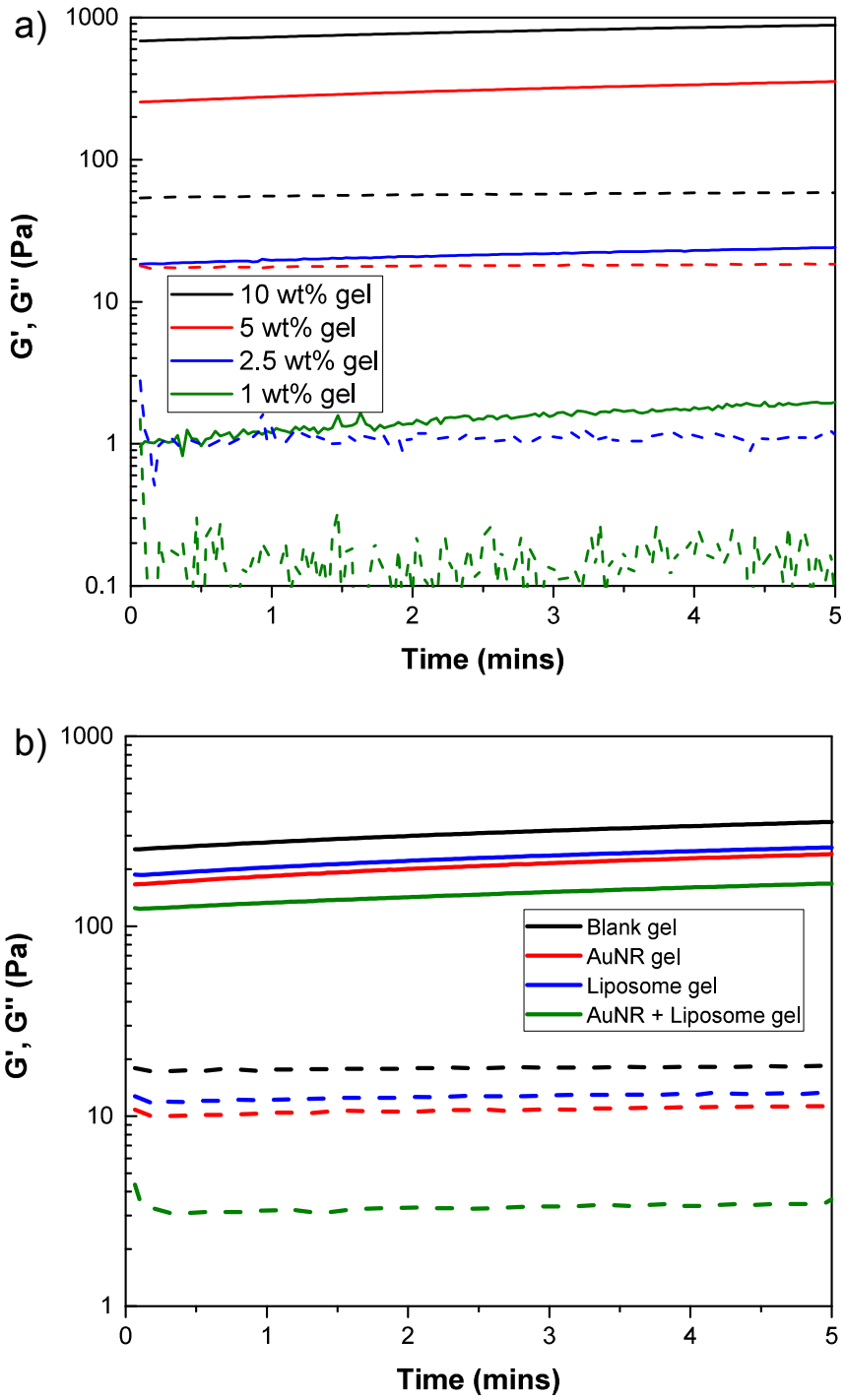

Figure S5. The storage moduli (solid lines) and loss moduli (dashed lines) of; a) PEG gels of 10 wt% (black), 5 wt% (red), 2.5 wt% (blue) and 1 wt% (green), b) 5 wt% gel (black) and 5 wt% gels containing AuNRs (red), liposomes (blue) and AuNRs with liposomes (green).

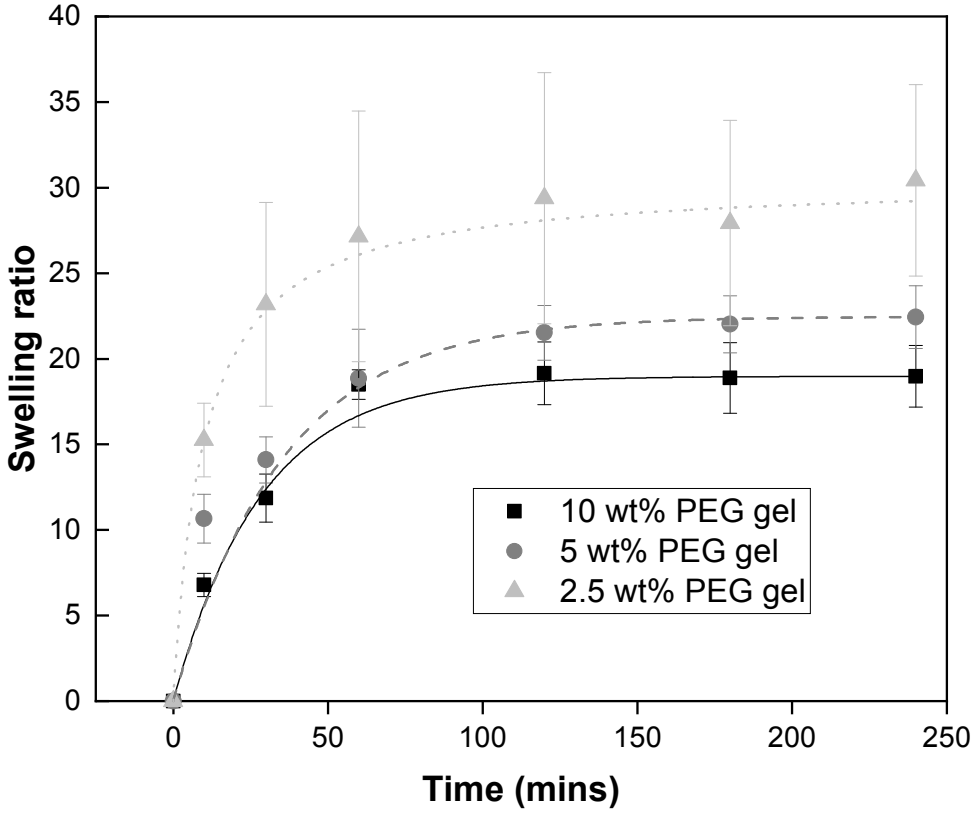

Figure S6. The change in the swelling ratio with time as freeze-dried gels of 10 wt% (black squares, solid line), 5 wt %(dark grey circles) and 2.5 wt% (light grey triangles, dotted line) were added to 1 ml of Milli-Q. Error bars indicate standard deviation (n=5).

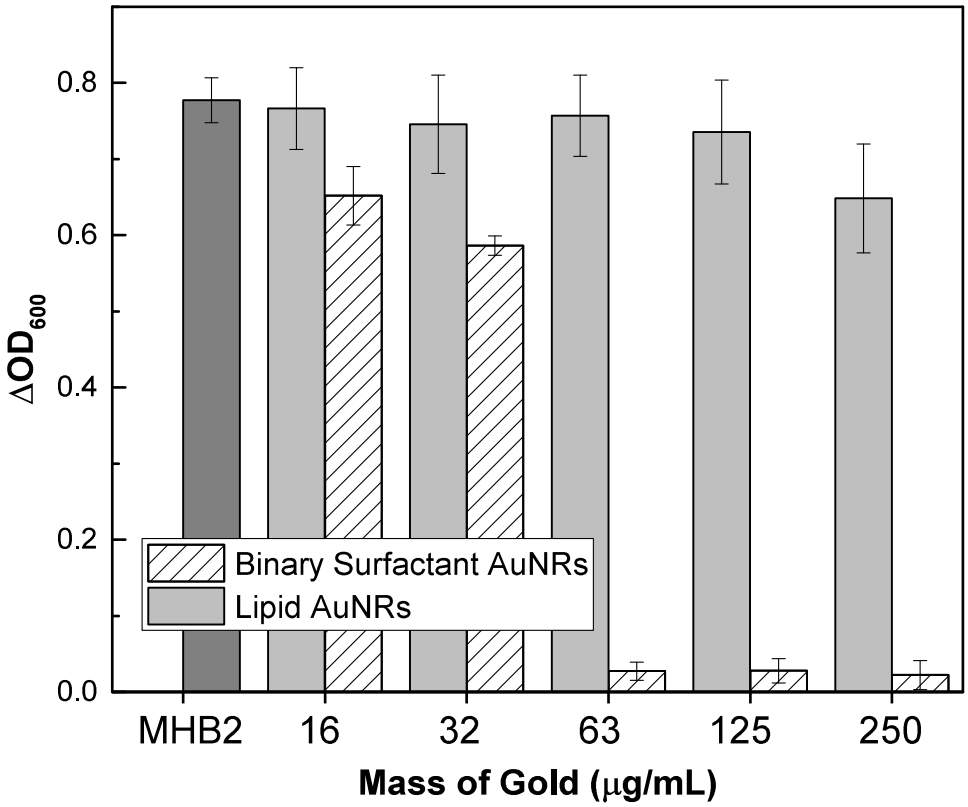

Figure S7. The change in optical density of S. aureus when incubated with increasing concentrations of CTAB-NaOL (diagonal slashed column), phospholipid (light grey column) stabilised AuNRs for 18 hours. Error bars indicate standard deviation (n=6).

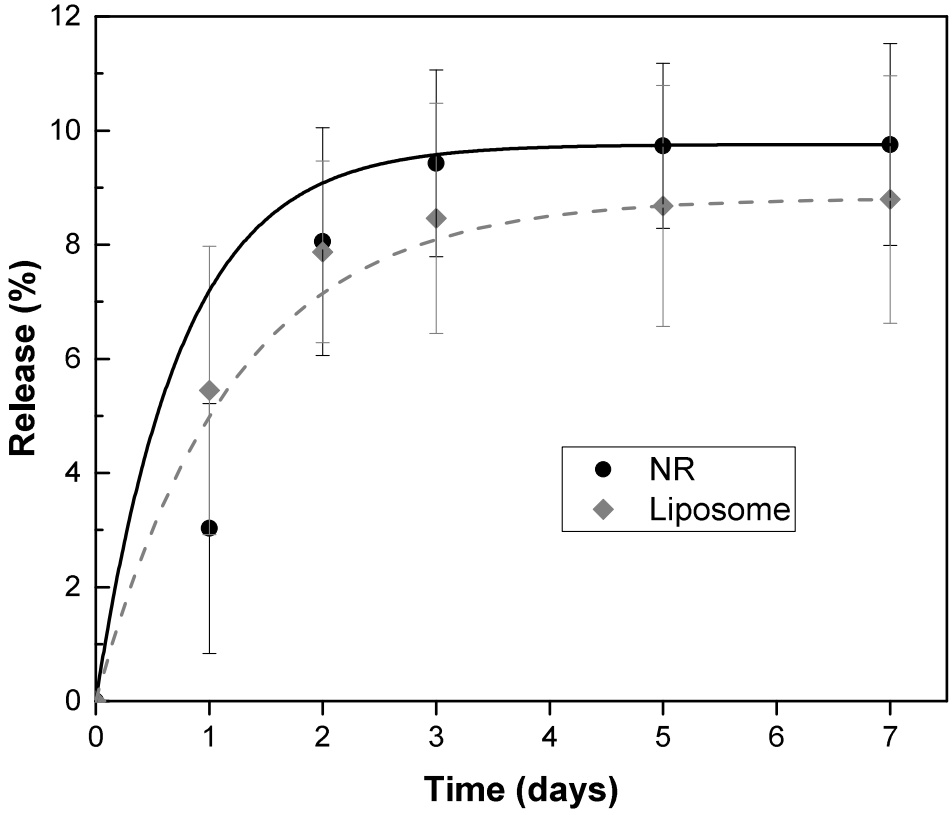

Figure S8. The release of lipid-coated AuNRs (black circles) and Texas-red labeled liposomes (grey diamonds) from a 50 µl 5 wt% PEG hydrogel into 150 µl of citrate buffer over a 7-day observation. (n=4).

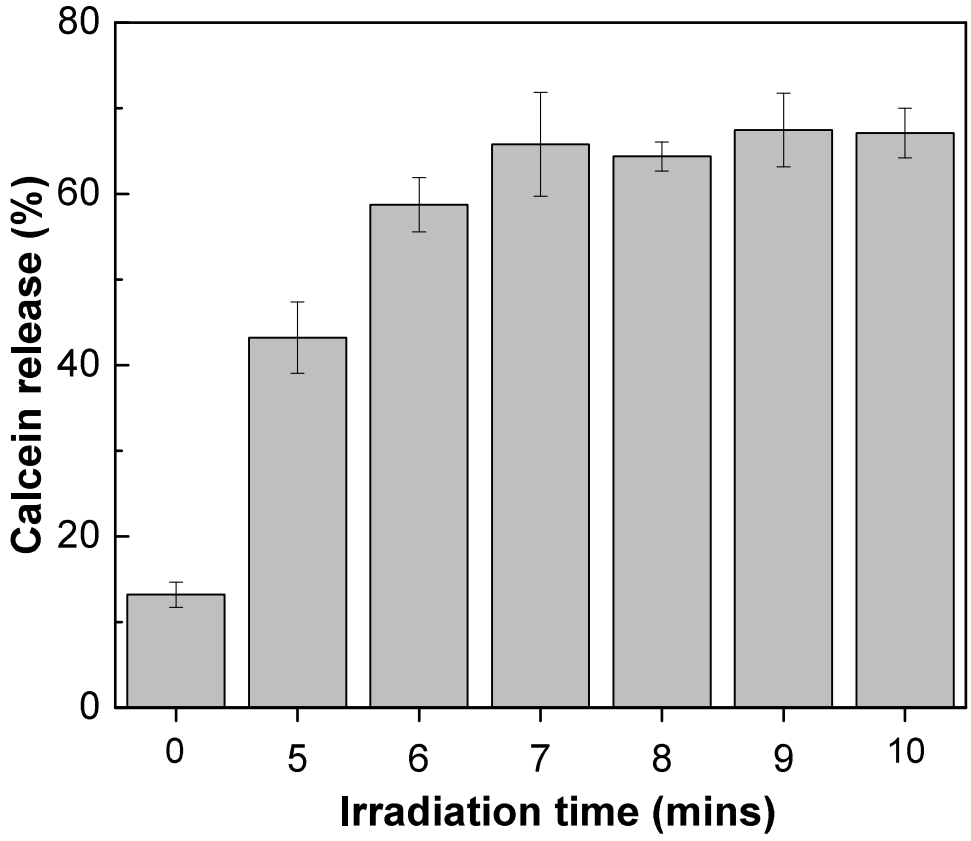

Figure S9. Triggered release of calcein from liposomes loaded into 5 wt% gels after incubation in a *S. aureus* suspension for 1 h before laser irradiation at 860 nm for 5-10 min, with a laser intensity of 2.1 W cm^{-2}. Fluorescence measurements (Exc/Em 496/515 nm) were taken immediately after addition to the bacteria suspension and after an 18 h incubation at 37°C. 1% Triton-X100 was added to the control wells to find the maximum fluorescence. Results are the average of six replicates.