
بسم الله الرحمن الرحيم

Multifunctional Nanoagents For Ultrasensitive Imaging And Photoactive Killing Of Gram-negative And Gram-positive Bacteria

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Multifunctional nanoagents for ultrasensitive imaging and photoactive killing of Gram-negative and Gram-positive bacteria

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Simultaneous imaging and treatment of infections remains a major challenge, with most current approaches being effective against only one specific group of bacteria or not being useful for diagnosis. Here we develop multifunctional nanoagents that can potentially be used for imaging and treatment of infections caused by diverse bacterial pathogens. The nanoagents are made of fluorescent silicon nanoparticles (SiNPs) functionalized with a glucose polymer (e.g., poly[4-O-(α-D-glucopyranosyl)-D-glucopyranose]) and loaded with chlorin e6 (Ce6). They are rapidly internalized into Gram-negative and Gram-positive bacteria by a mechanism dependent on an ATP-binding cassette (ABC) transporter pathway. The nanoagents can be used for imaging bacteria by tracking the green fluorescence of SiNPs and the red fluorescence of Ce6, allowing in vivo detection of as few as 10^5 colony-forming units. The nanoagents exhibit in vivo photodynamic antibacterial efficiencies of 98% against *Staphylococcus aureus* and 96% against *Pseudomonas aeruginosa* under 660 nm irradiation.

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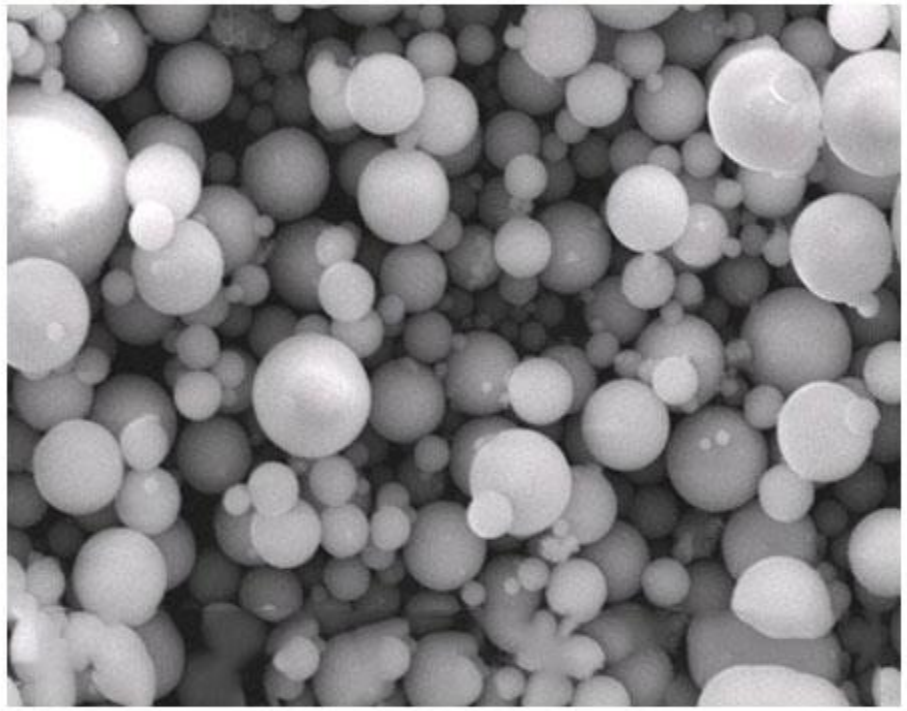
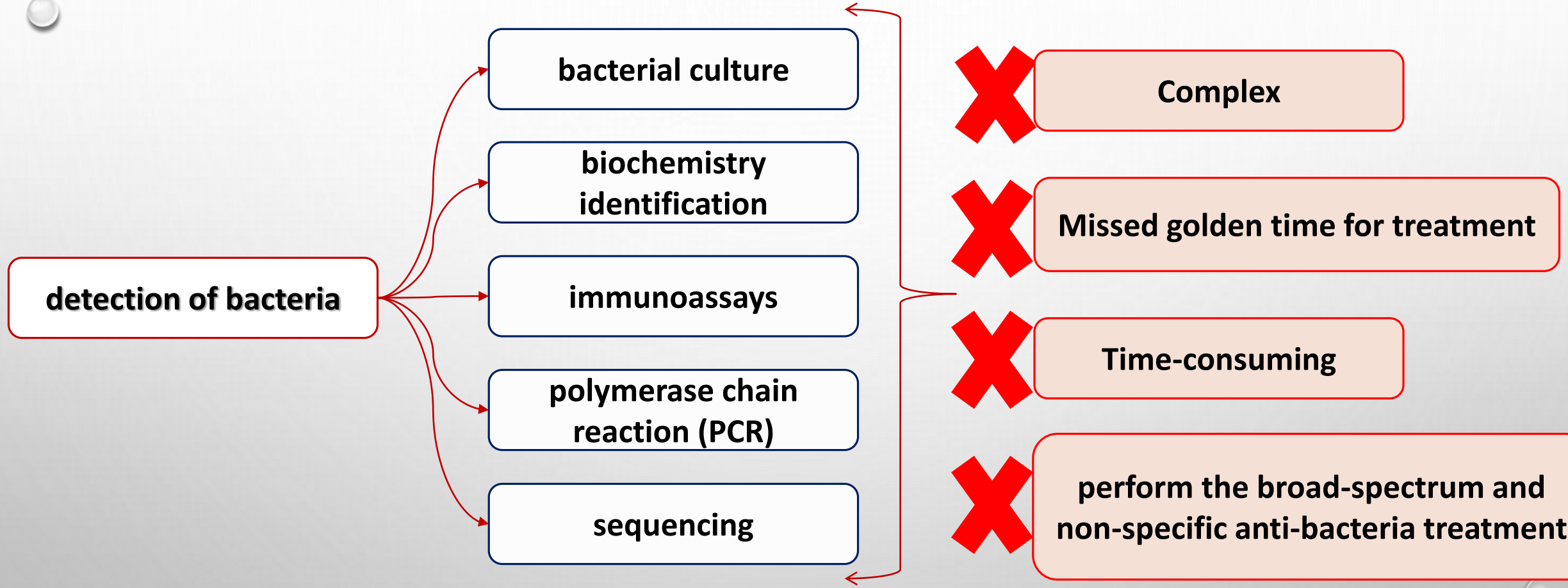
10. *Discussion*

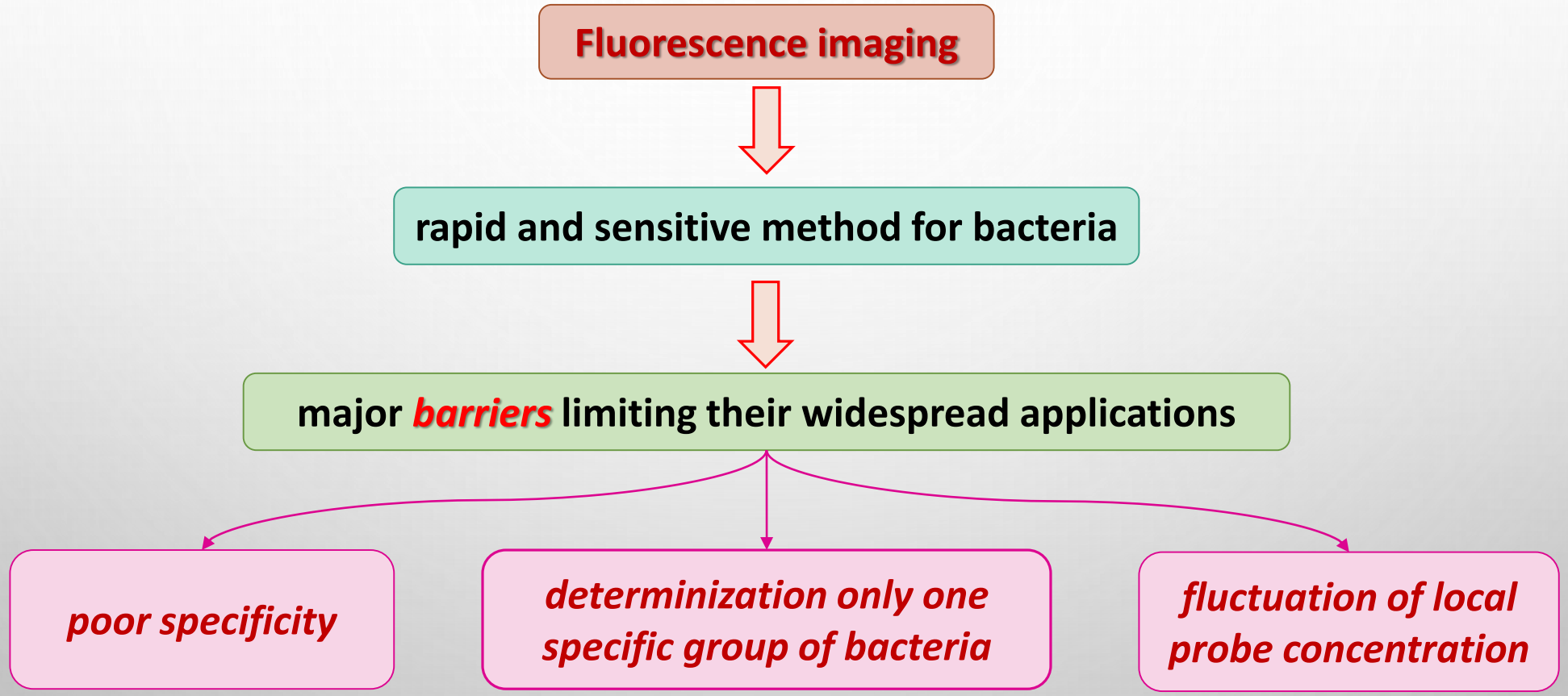


Silicon Dioxide

Introduction



Rapid, sensitive, specific, and reasonably priced diagnostics for effective treatments



GP-CE6-SiNPs advantages

- Benign Biocompatibility
- glucose polymer (GP) as the major microbial carbon source
- Bright Fluorescence
- Adjustable Drug Loading Capacity
- Strong Photostability
- Ultrasmall SiNPs (<10 nm) approved by FDA

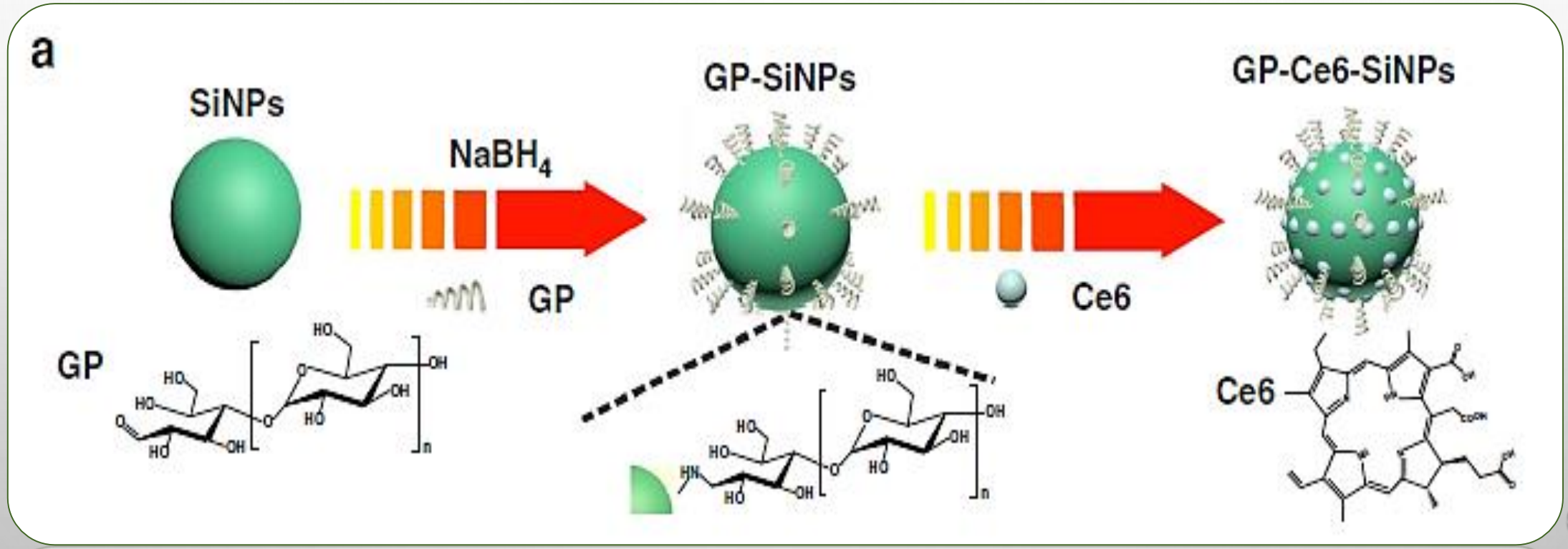
chlorin e6 (Ce6), is able to provide stable red fluorescence signals as well as a photosensitizer, kill bacterial Cells

The antibacterial efficiency of the developed nanoagents is up to ca. 98% against S. aureus and ca. 96% against P. aeruginosa under 40-min exposure with a relative low power laser (660 nm).

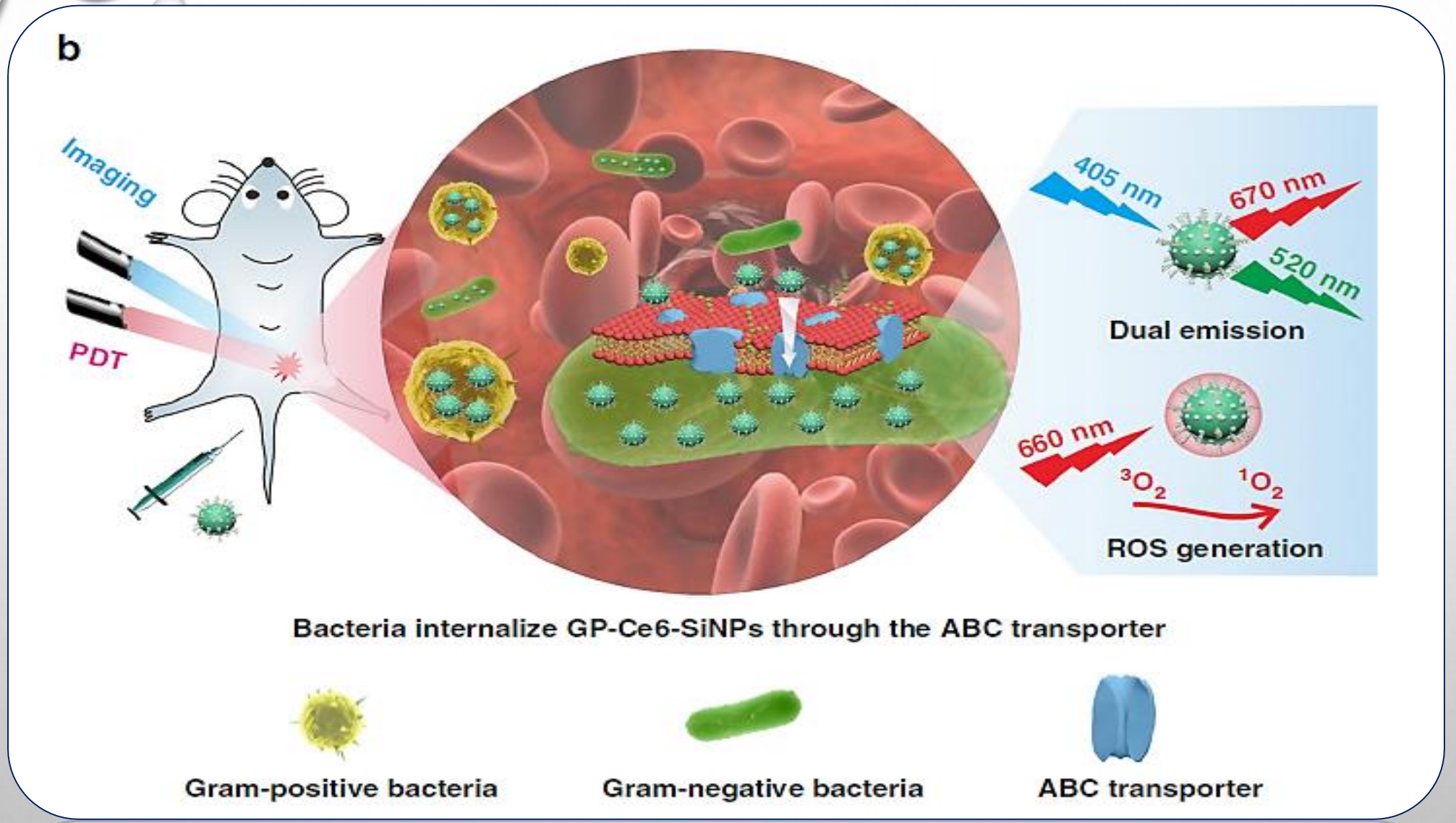
Methods

1. Fabrication of GP-CE6-SiNPs
2. Preparation of VAN-SiNPs
3. Bacterial culture
4. Fluorescence imaging of bacteria and cells in vitro
5. Quantifying transport of nanoagents into bacteria
6. In vivo imaging of bacterial infections
7. In vitro antibacterial assays
8. In vivo antibacterial assays
9. In vitro and in vivo toxicity assessment

Design of multifunctional nanoagents for detection and photodynamic treatment of bacterial infections

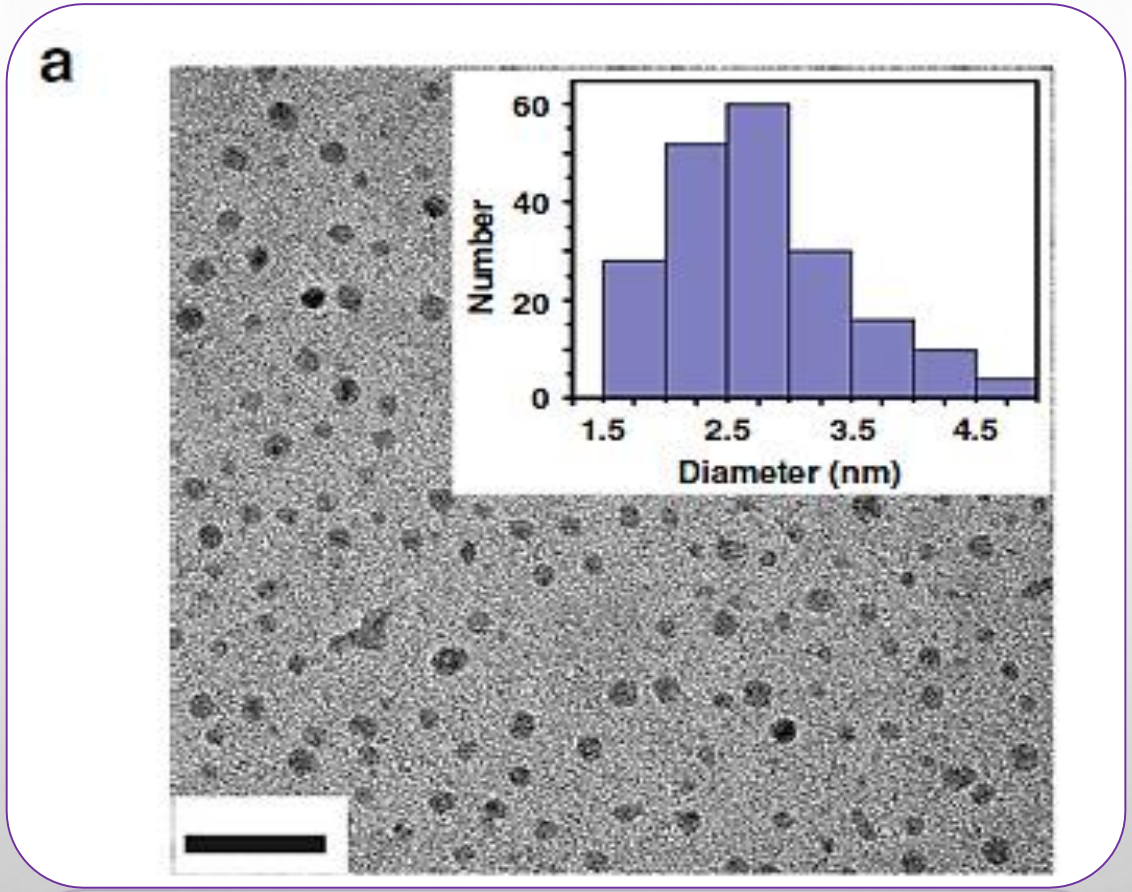


a) Synthetic route of nanoagents of GP-Ce6-SiNPs. GP-Ce6-SiNPs are composed of SiNPs conjugated to GP and loaded with Ce6 molecules



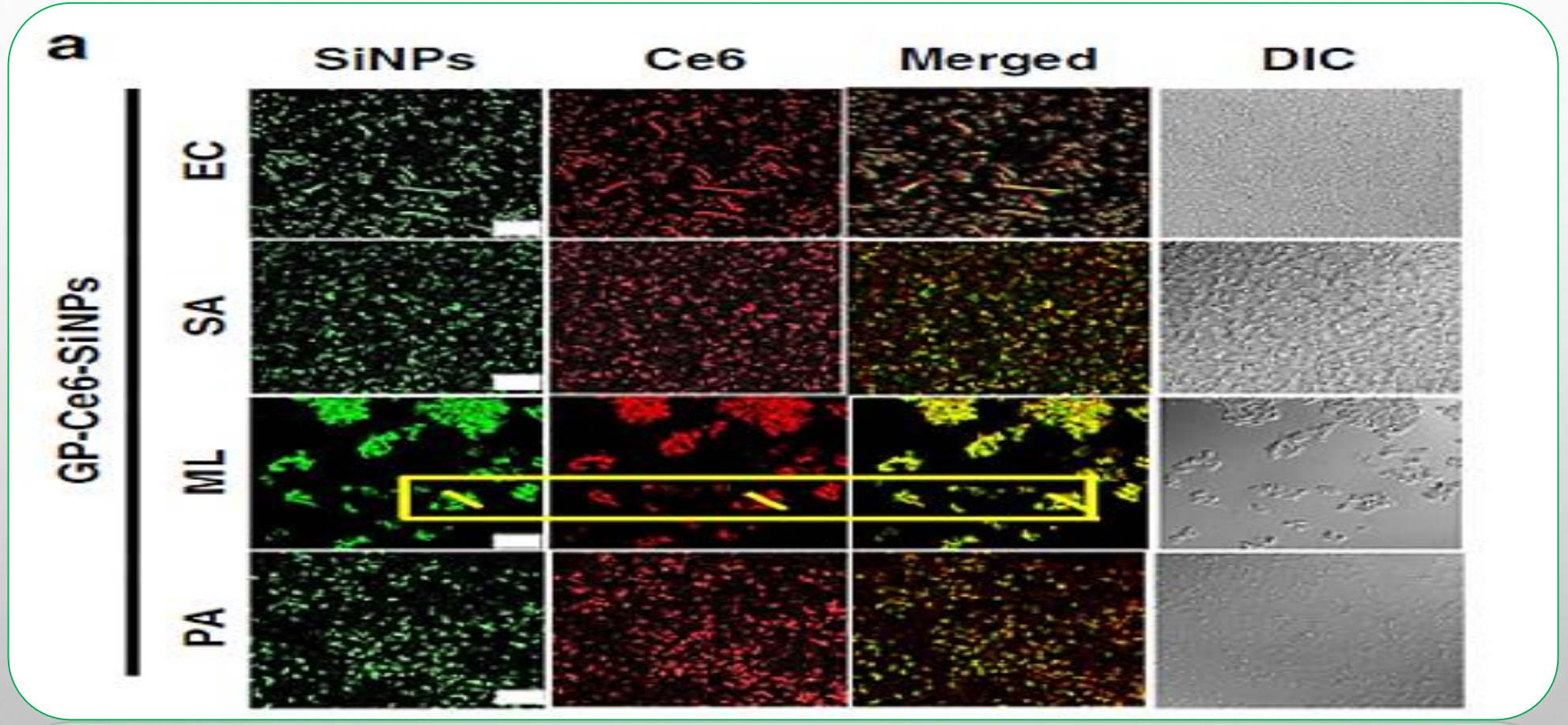
b) Imaging and treatment of Gram-negative and Gram positive bacterial infections by GP-Ce6-SiNPs. GP-Ce6-SiNPs are robustly internalized by ABC transporters, which are only present in bacterial cells while not present in mammalian cells.

Characterization of GP-CE6-SINPS

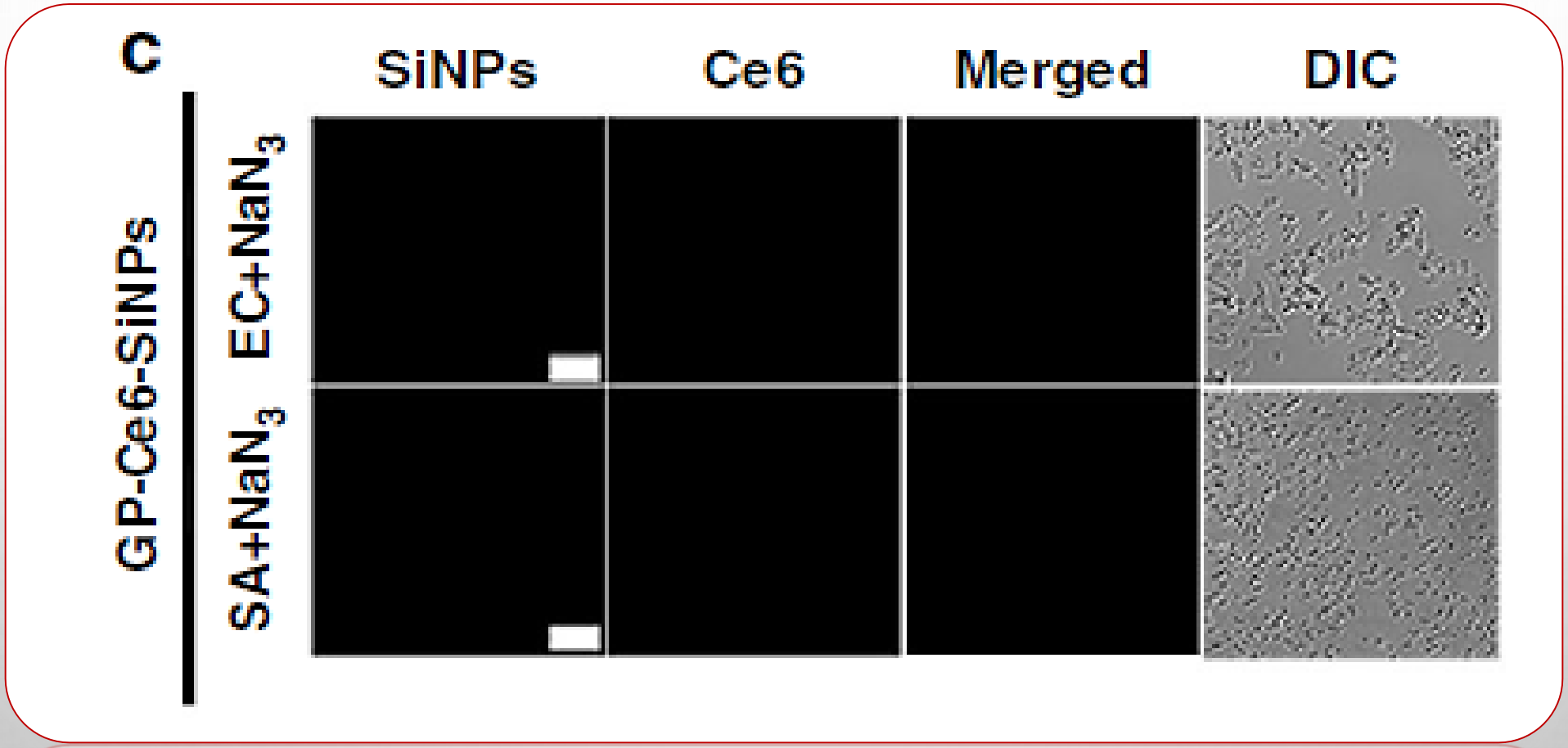


a) TEM image and corresponding size distribution (inset) of GP-Ce6-SiNPs. Scale bar: 20 nm.

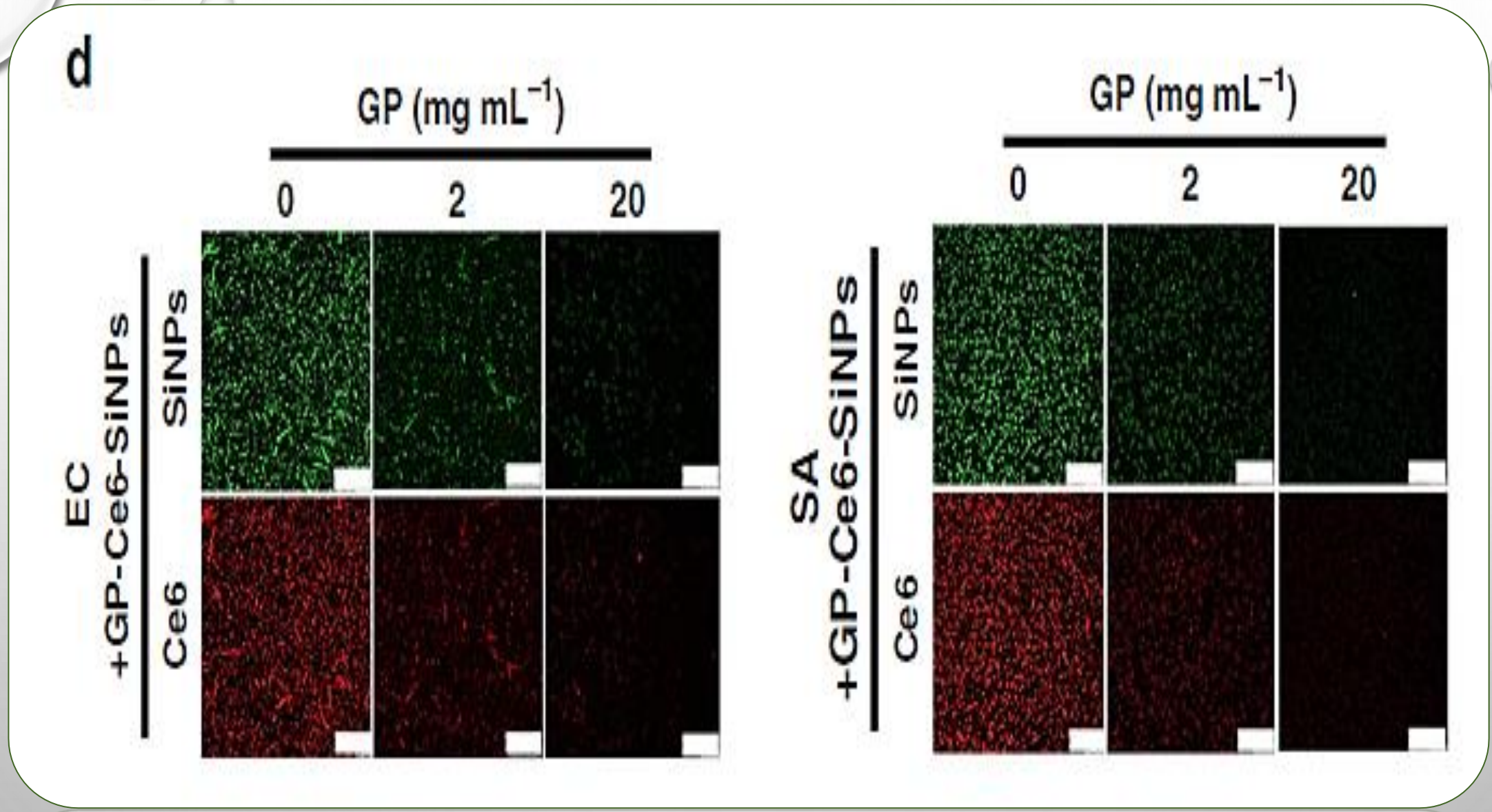
In vitro imaging of gram-negative and gram-positive bacteria



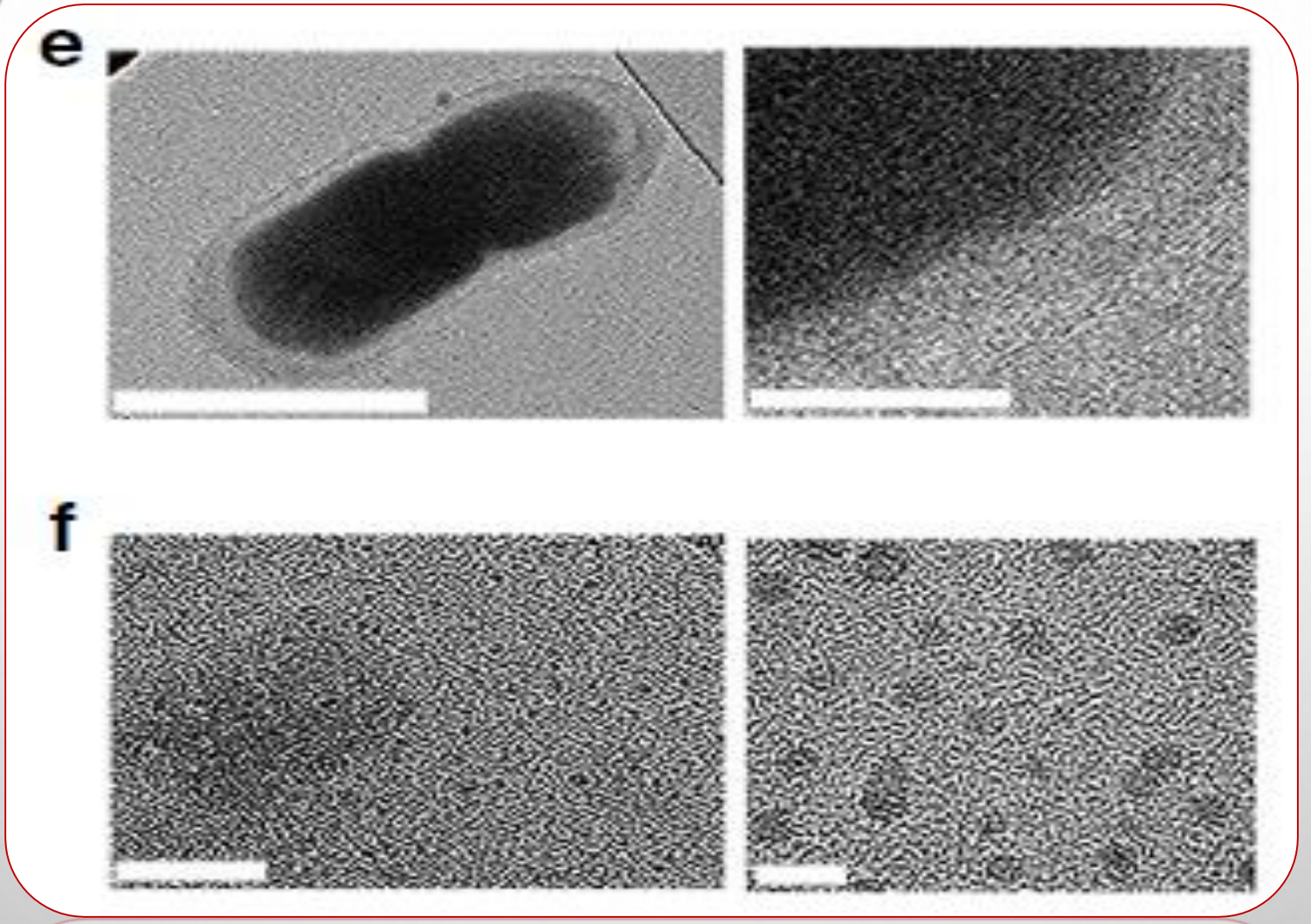
a) Confocal fluorescence images of four different kinds of bacteria (EC, SA, ML, and PA) after incubation with GP-Ce6-SiNPs. Scale bar: 10 µm.



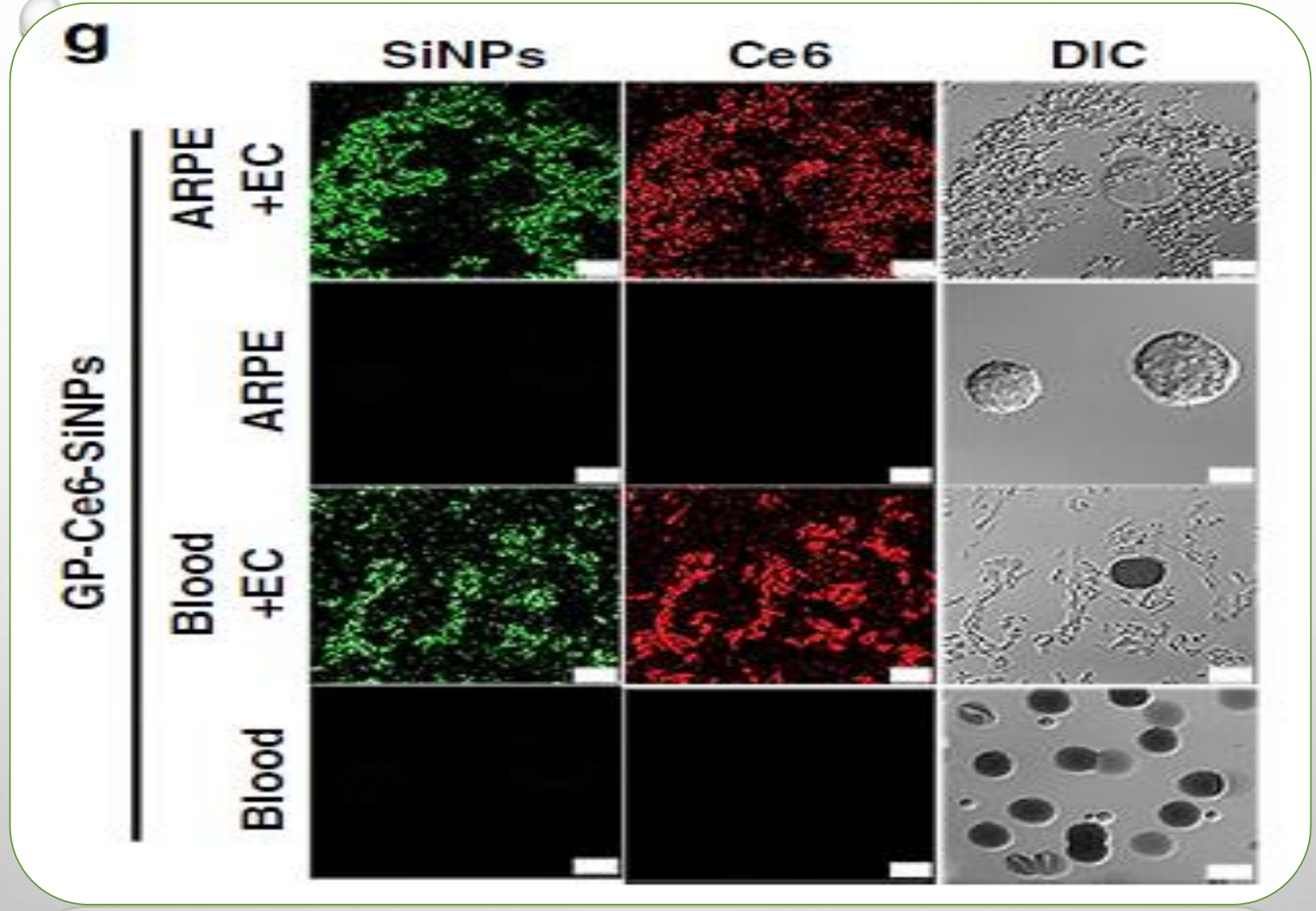
c) Confocal fluorescence images of EC and SA treated with NaN3 and then incubated with GP-Ce6-SiNPs for 2 h. Scale bar: 10 μm.



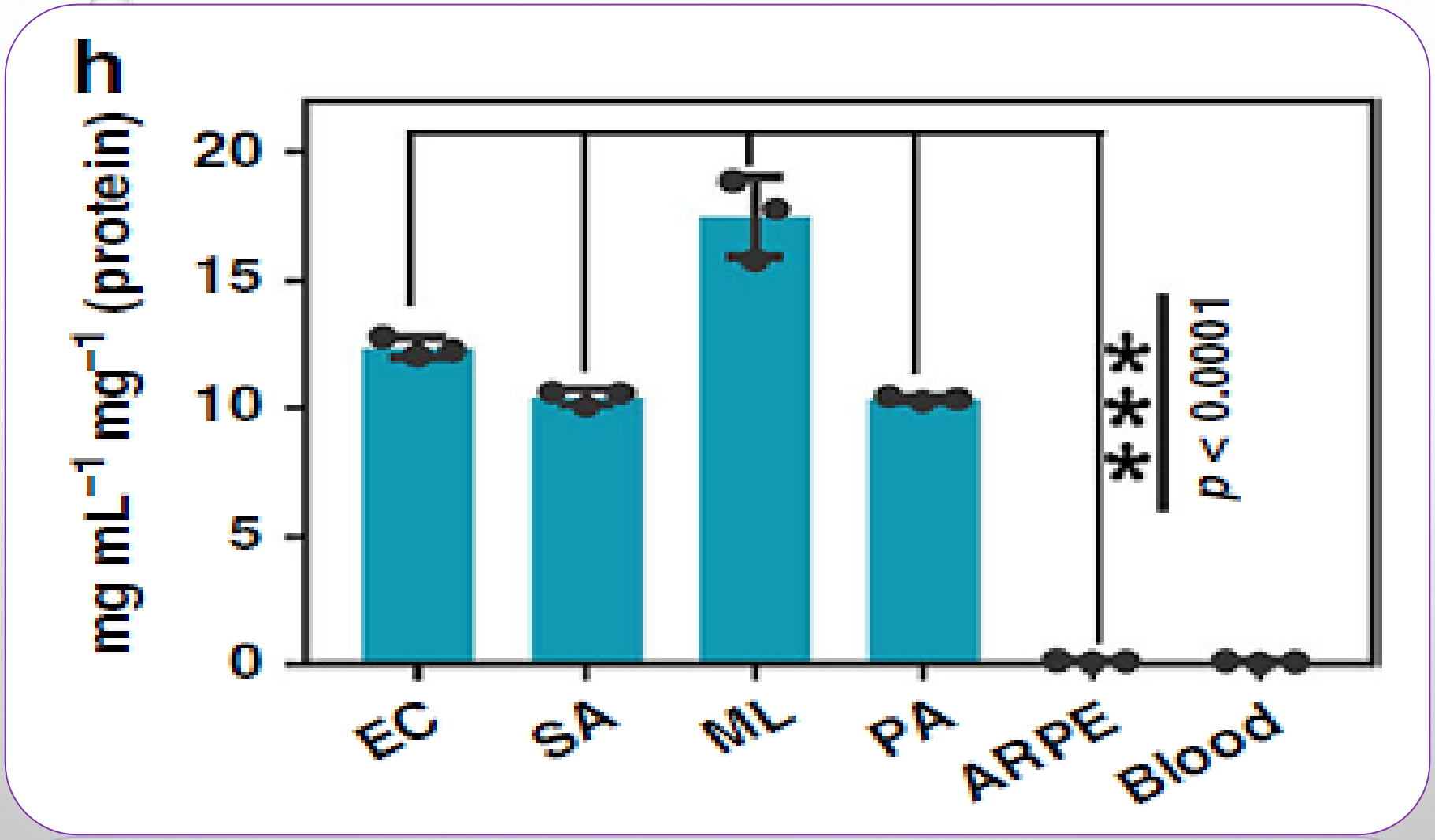
d) Confocal fluorescence images of EC and SA incubated with GP with different concentrations (0, 2, 20 mg mL−1) for 5 min and then incubated with GP-Ce6-SiNPs for 2 h. Scale bar: 25 µm.



e) TEM (scale bar: 1 µm) and zoom-in TEM (scale bar: 30 nm) images of intact EC and cell membrane of EC treated with GP-Ce6-SiNPs. f) TEM (scale bar: 20 nm) and zoom-in TEM (scale bar: 5 nm) images of GP-Ce6-SiNPs nanoparticles in cell lysate of EC.

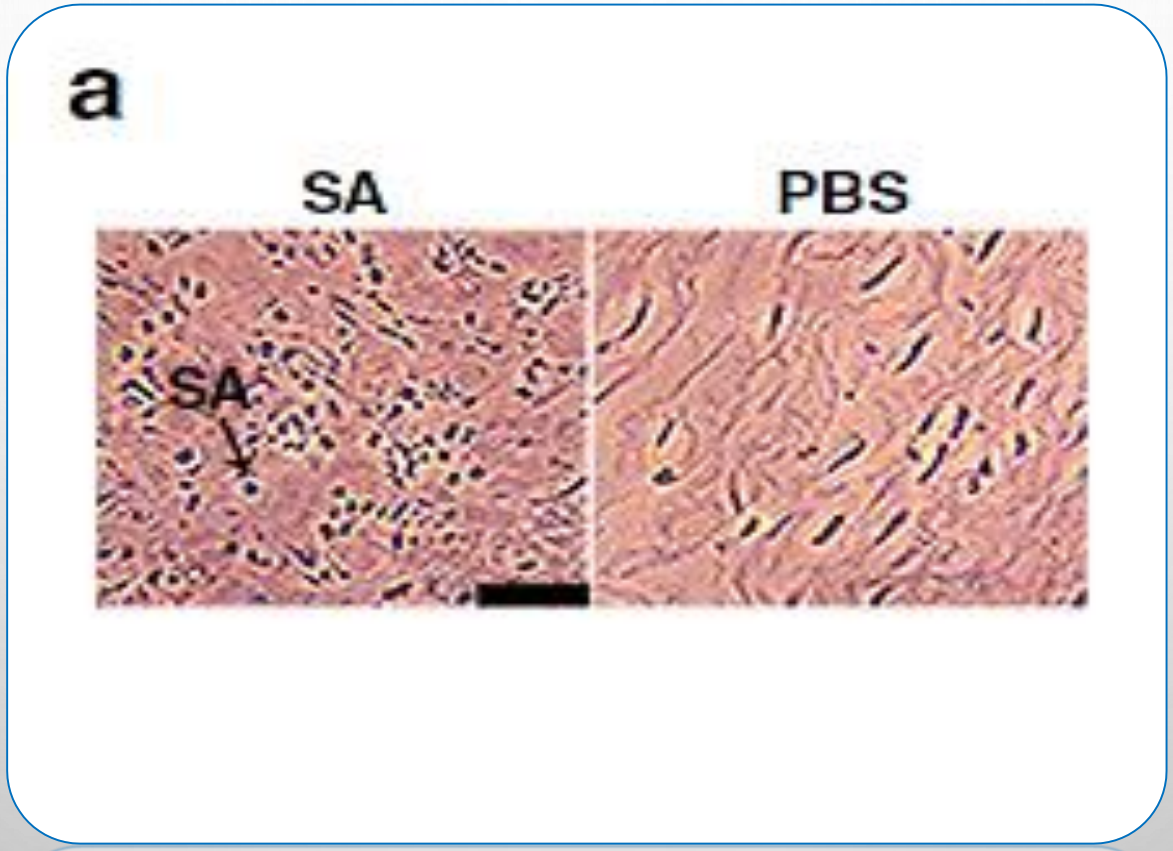


g) Confocal fluorescence images of the mixture of ARPE cells and EC, pure ARPE cells, the mixture of human blood and EC, pure human blood after incubation with GP-Ce6-SiNPs. Scale bar:25 μm.

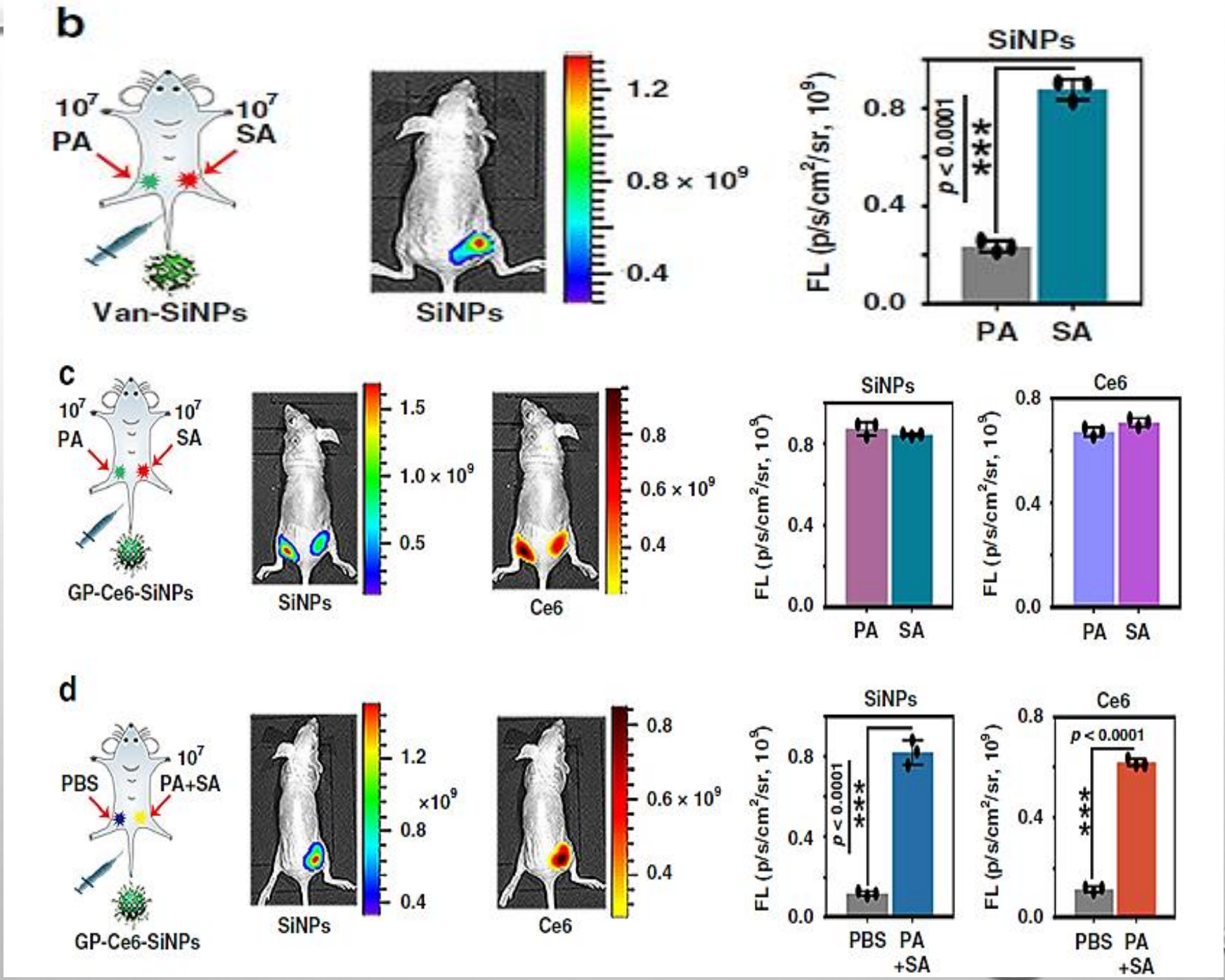


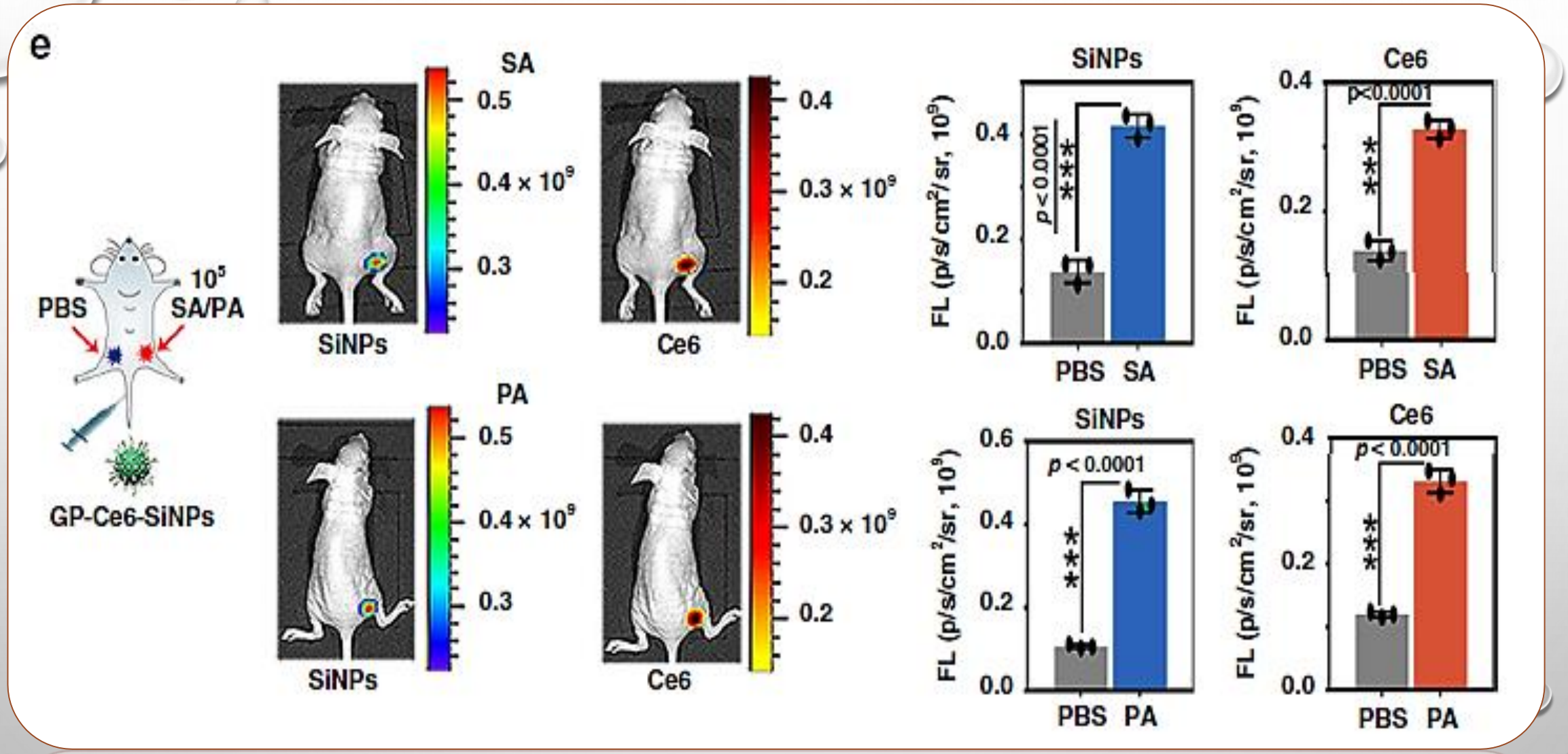
h) Histogram quantifying the level of GP-Ce6-SiNPs transport. Statistical analysis was performed using a one-way ANOVA analysis. Error bars represent the standard deviation obtained from three independent measurements (*p < 0.001, n =3). In the above experiments, the final concentration of GP-Ce6-SiNPs is 10 mg mL–1 (3.6 mg mL–1 of GP and 100 µgmL–1 of Ce6).**

In vivo imaging of infections caused by gram-negative and gram-positive bacteria

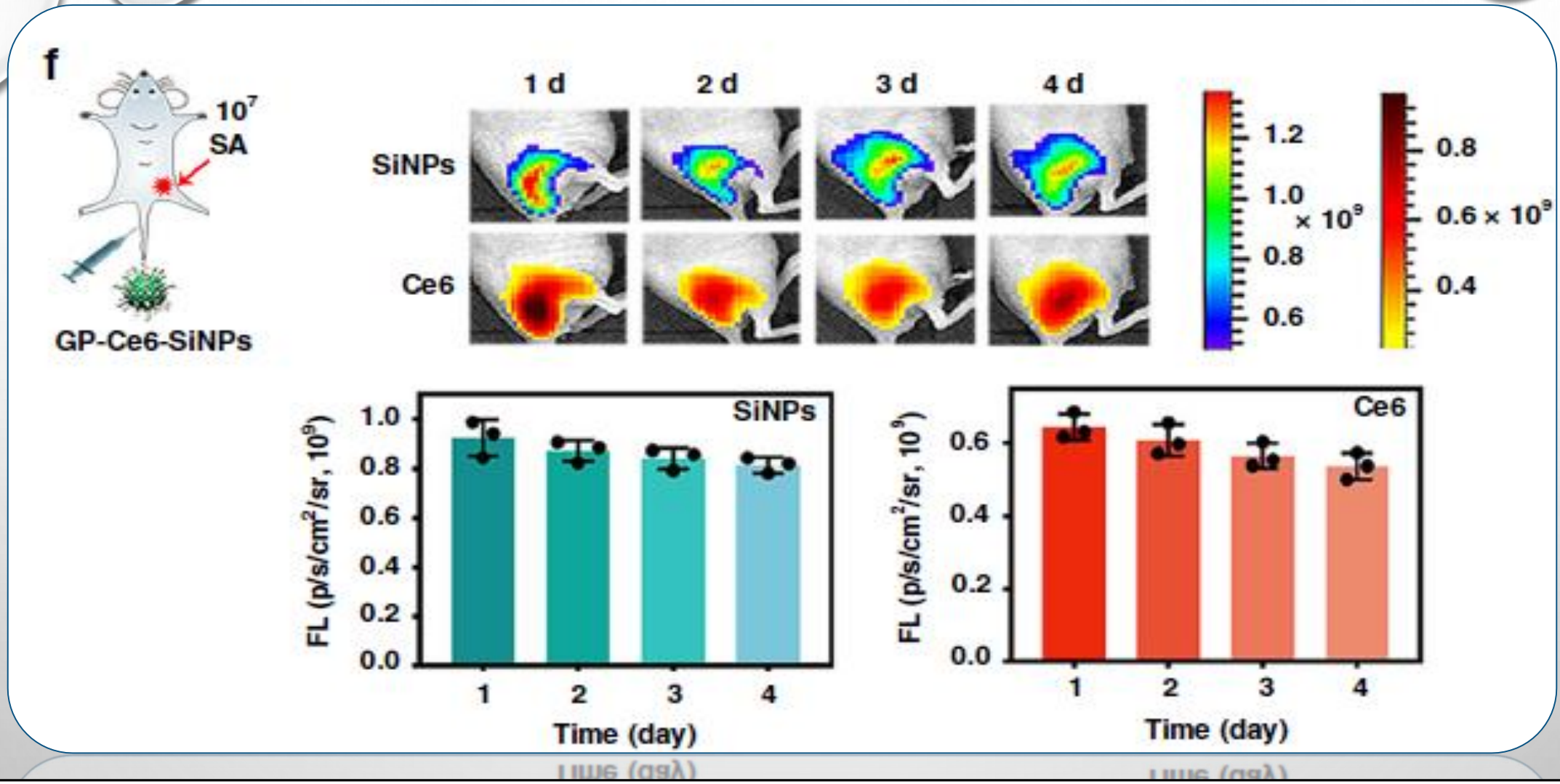


a) Micrograph of the histology of 1.0 × 107 CFU of SA-infected and PBS-treated muscles. Scale bar: 50 µm.


b
10^7 PA
10^7 SA
Van-SiNPs
SiNPs
1.2
0.8 × 10^9
0.4
SiNPs
FL (p/s/cm²/sr, 10^9)
0.8
0.4
0.0
p < 0.0001
***
PA
SA
c
10^7 PA
10^7 SA
GP-Ce6-SiNPs
SiNPs
1.5
1.0 × 10^9
0.5
Ce6
0.8
0.6 × 10^9
0.4
SiNPs
Ce6
FL (p/s/cm²/sr, 10^9)
PA
SA
d
PBS
10^7 PA+SA
GP-Ce6-SiNPs
SiNPs
1.2
×10^9
0.8
0.4
Ce6
0.8
0.6 × 10^9
0.4
SiNPs
Ce6
FL (p/s/cm²/sr, 10^9)
p < 0.0001
***
PBS
PA +SA

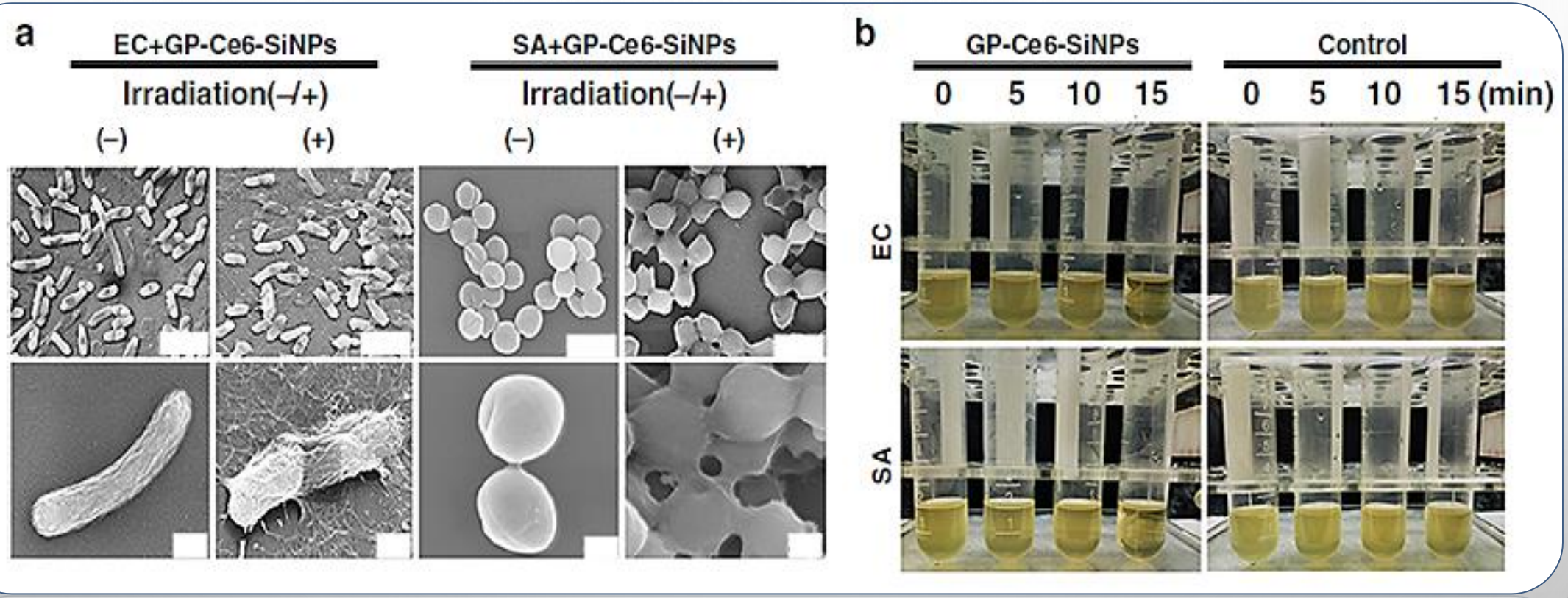


e) In vivo dual-emission imaging of 1.0 × 105 CFU of SA or PA (right side) and PBS (left side)-treated sites of mice injected with GP-Ce6-SiNPs and corresponding histograms of fluorescence intensity at two sites.



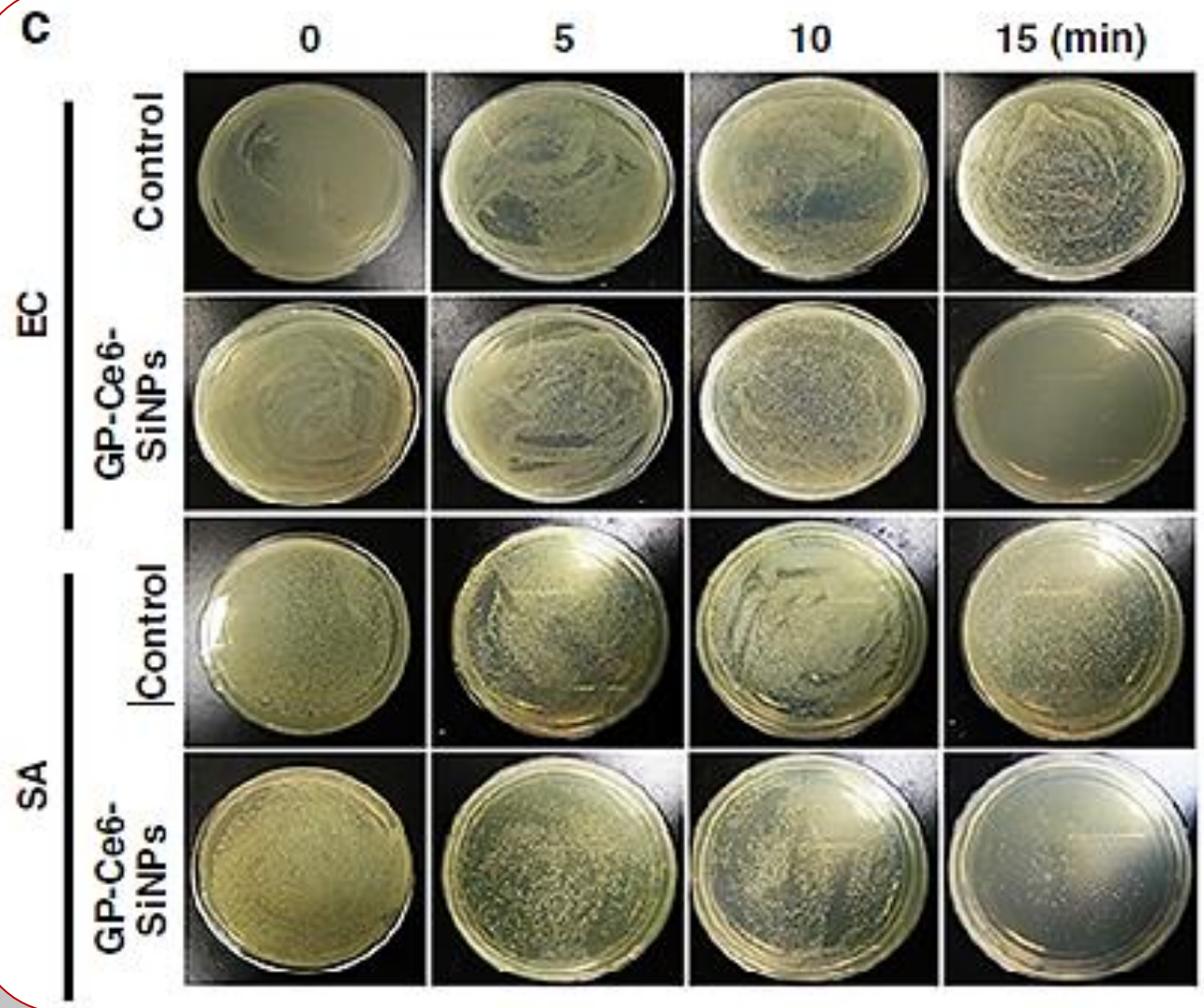
f) Long-term in vivo dual-emission imaging of 1.0 × 107 CFU of SA-infected site of mice injected with GP-Ce6-SiNPs and corresponding time-dependent histograms of fluorescence intensity. The amount of bacteria at the infection site during imaging is determined via tissue harvesting, homogenization and culturing with CFU count. Statistical analysis was performed using paired two-tailed t-test. Error bars represent the standard deviation obtained from three independent measurements (*p < 0.001, n = 3). The number (n) of mice in each experiment is 3, the total number is 18, and the gender of all mice is female.**

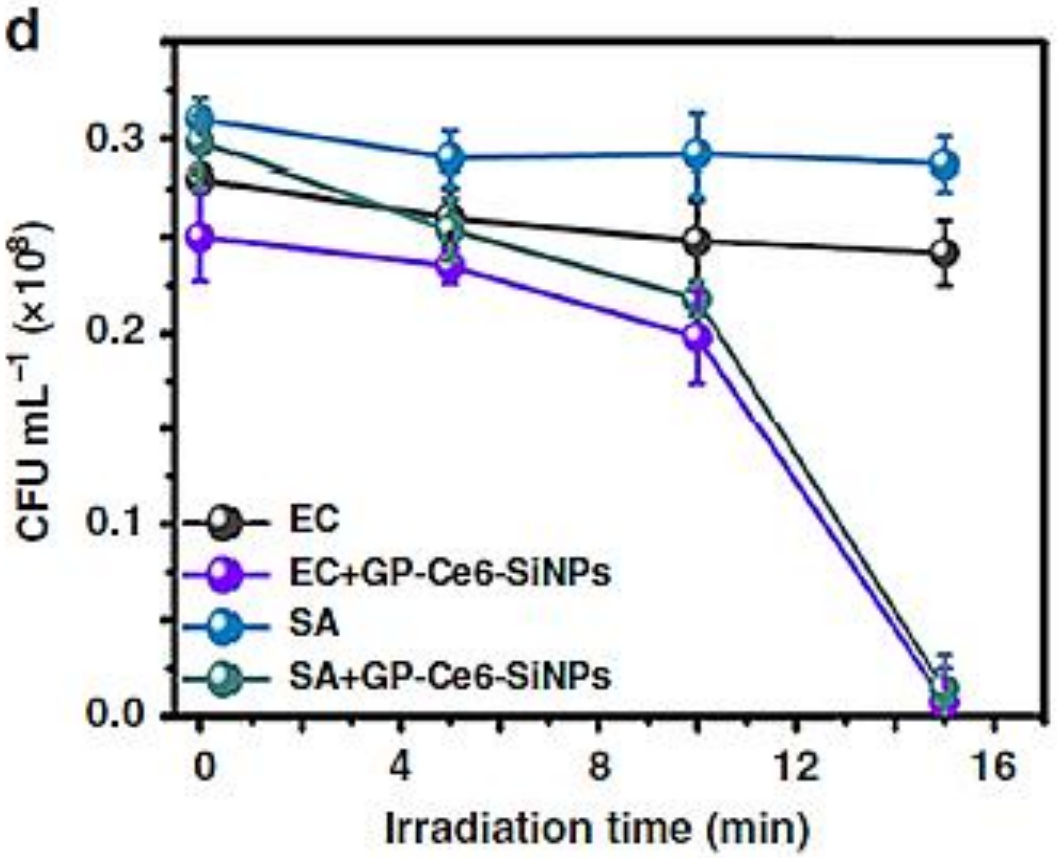
In vitro antibacterial activity of GP-CE6-SINPS



a) SEM images of GP-Ce6-SiNPs-treated EC and SA before and after constant 660-nm irradiation (12 mWcm−2) for 15 min (scale bar in the first row: 1 μm, scale bar in the second row: 200 nm).

b) The turbidity of EC and SA suspensions treated without GPCe6- SiNPs (control groups) or with GP-Ce6-SiNPs under constant irradiation (660 nm, 12mWcm−2) for 0, 5, 10, and 15 min. The irradiation treatment on each sample is performed for one time.

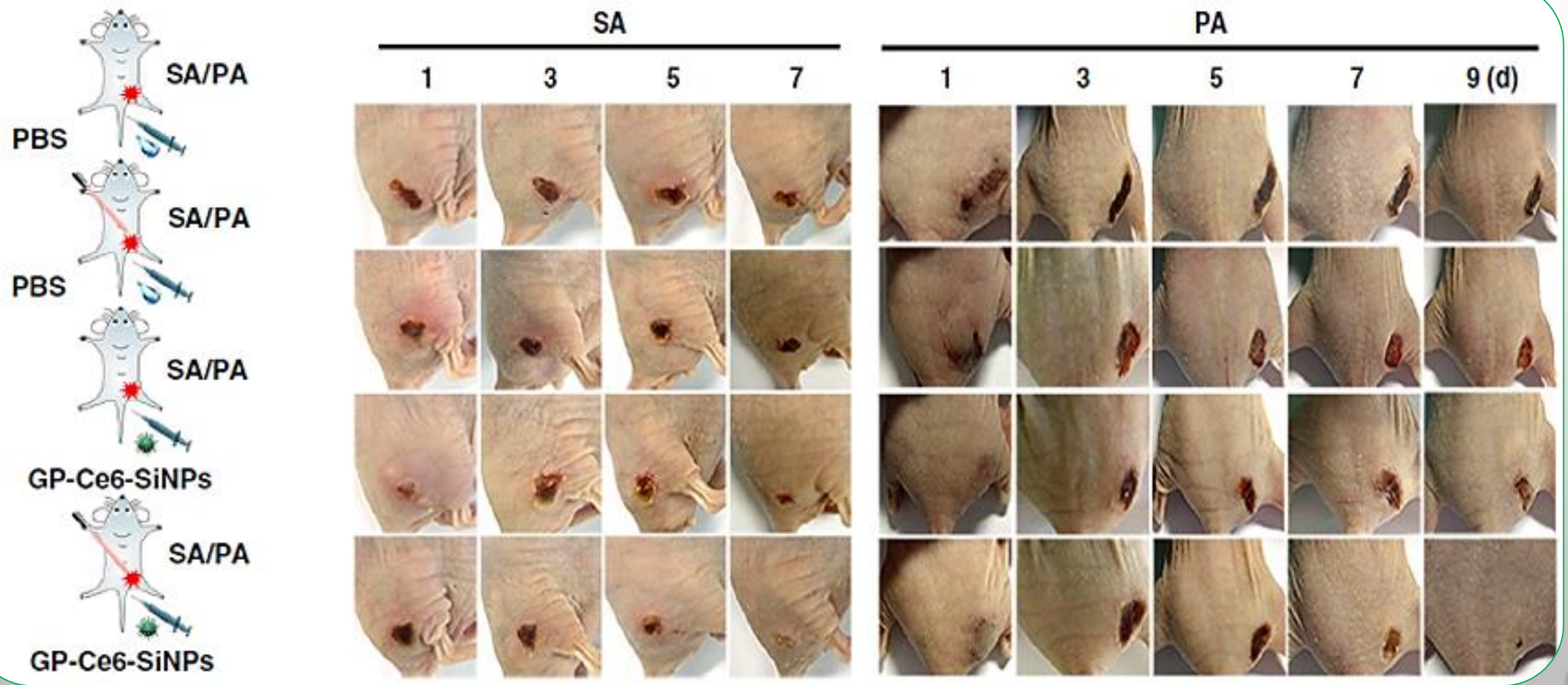




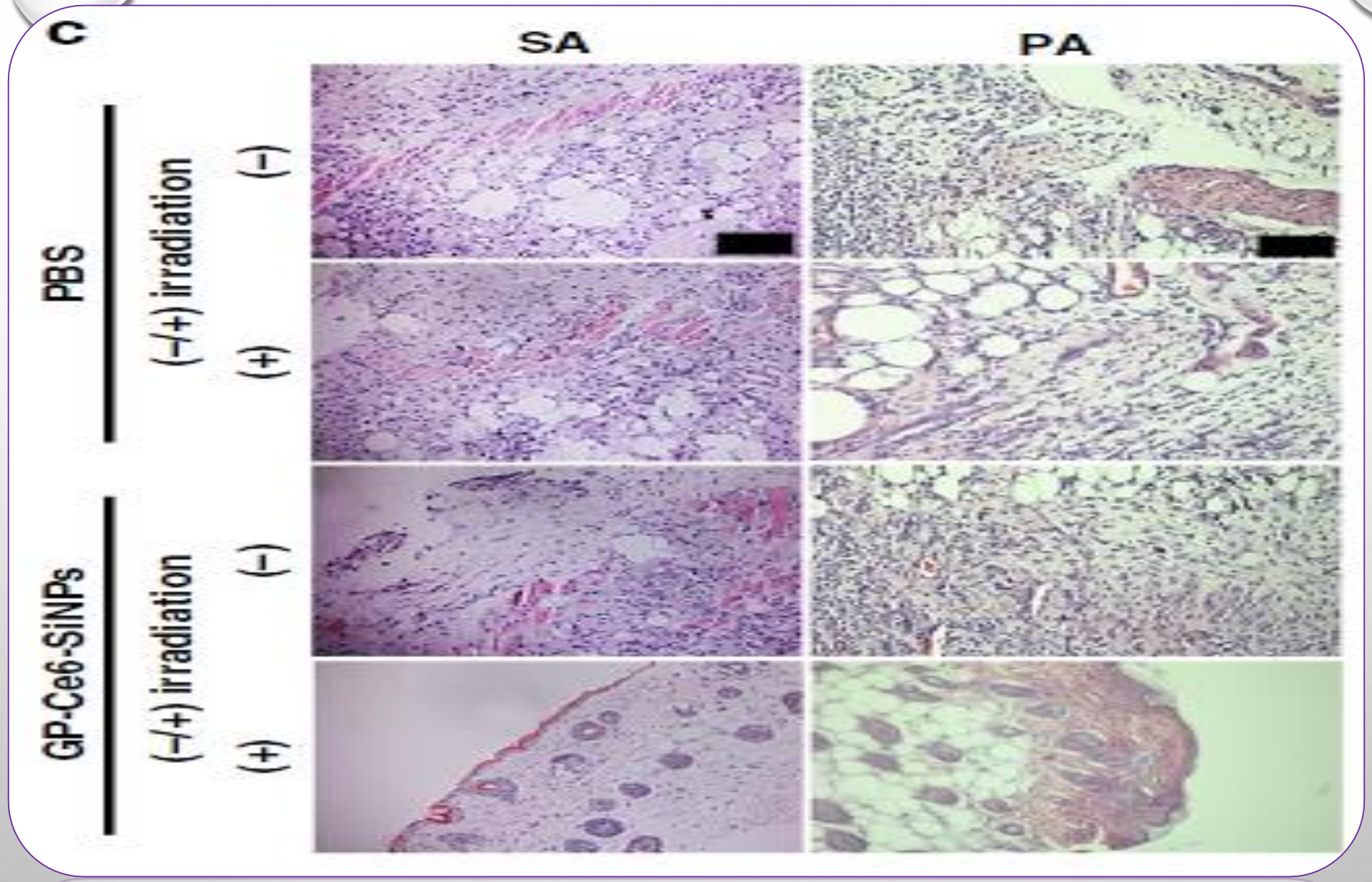
c) Photographs of the agar plates of EC and SA treated without (control groups) or with GP-Ce6-SiNPs under constant irradiation (660 nm, 12mWcm−2) for 0, 5, 10, and 15 min.

d) The corresponding bacterial counts (CFU mL−1) in panel (c). Error bars represent the standard deviation obtained from three independent measurements. The concentration of GP-Ce6-SiNPs is 10 mg mL−1 (3.6 mg mL−1 of GP and 100 μgmL−1 of Ce6).

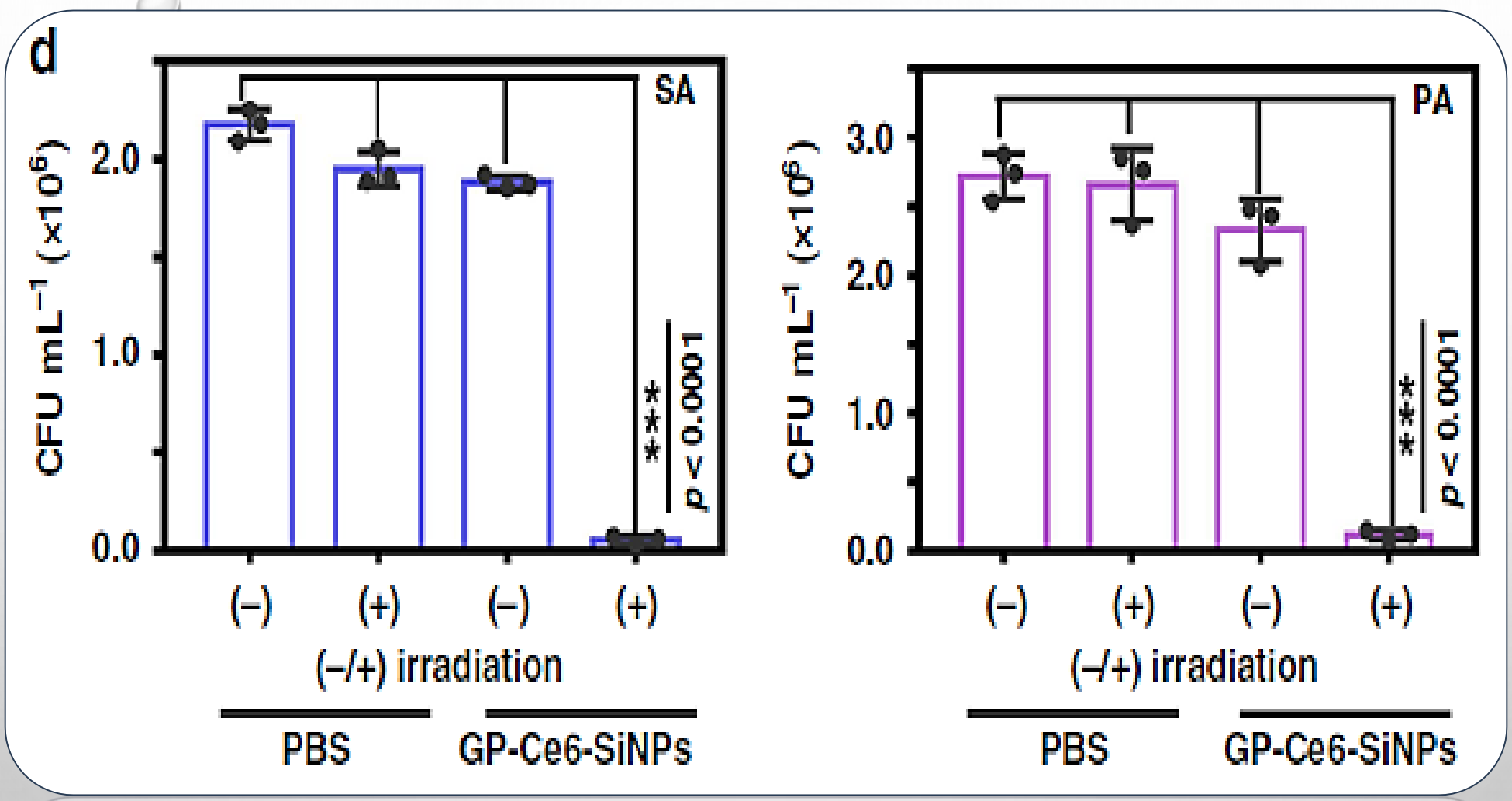
In vivo antibacterial activity of GP-CE6-SINPS



a) Representative photographs of time-dependent of SA- and PA-infected mice injected with GP-Ce6-SiNPs or PBS treated with or without light irradiation (660 nm, 12mWcm−2) for constant 40 min.

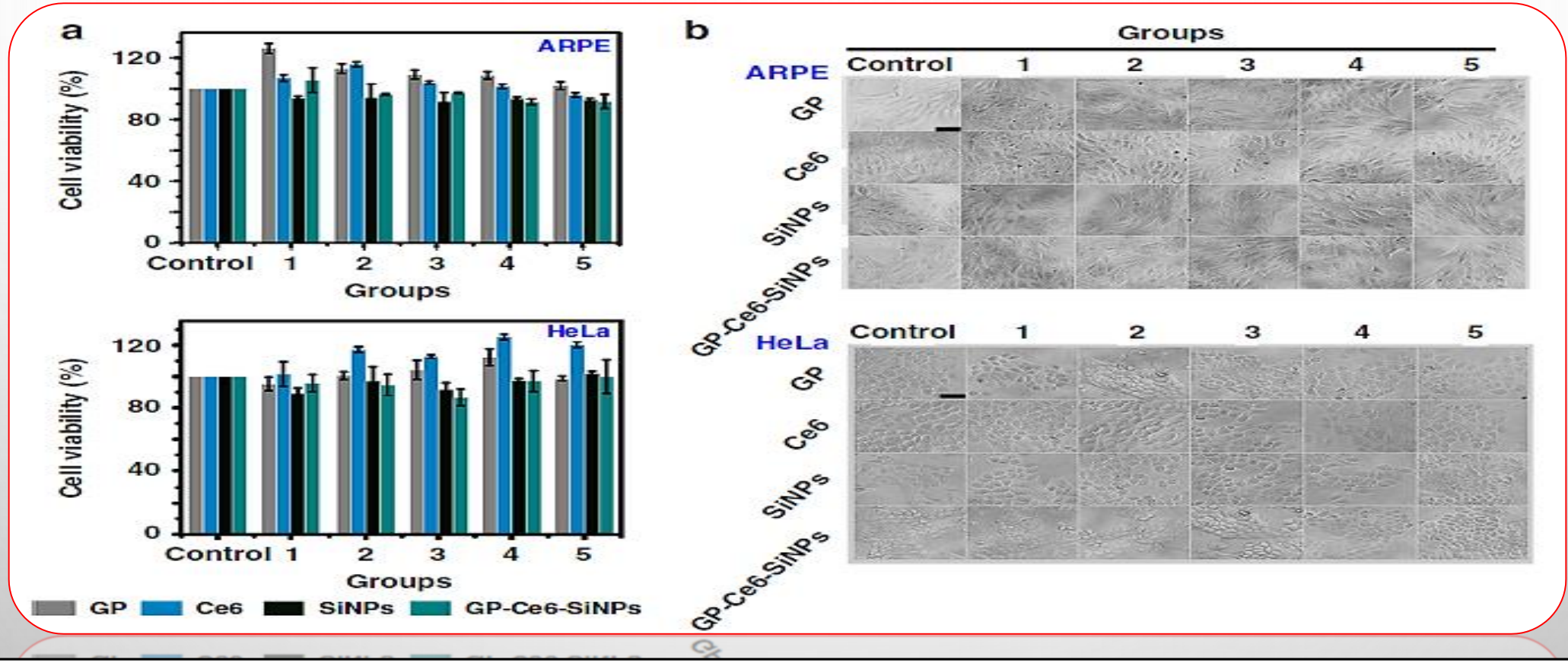


c) Corresponding histological images of the SA- and PA-infected skin tissues of mice in panel (a) at the 7th (SA) and 9th day (PA) post injection. Scale bar: 100 µm.

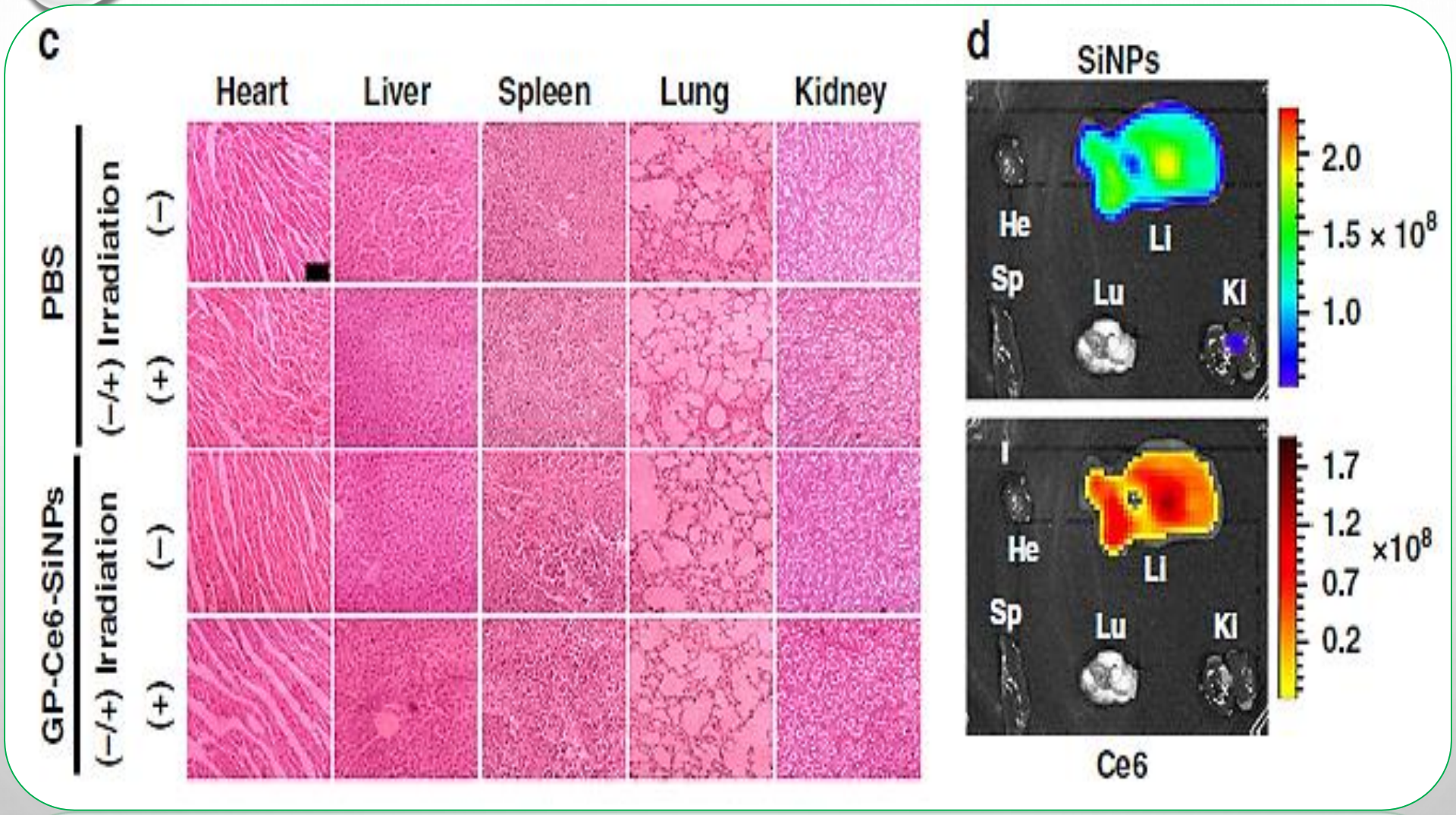


d) Bacterial counts (CFU mL−1) excised from the SA- and PA-infected tissues of mice at the 7th (SA) and 9th (PA) day post injection. Statistical analysis was performed using a one-way ANOVA analysis. Error bars represent the standard deviation obtained from three independent measurements (*p < 0.05, **p < 0.01, ***p < 0.001, n =3). The concentration of GP-Ce6-SiNPs is 10mgmL−1 (3.6 mg mL−1 of GP and 100 µg mL−1 of Ce6). Typically, the number (n) of mice used per experiment is 3, the total number is 24, and the gender of mice is female.

In vitro and In vivo toxicity tests



Cell viability (a) and morphology (b) of ARPE (up, normal cells) and HeLa cells (down, cancer cells) treated with different dosages of GP, Ce6, SiNPs, and GP-Ce6-SiNPs for 24 h, respectively. The groups of "Control, 1, 2, 3, 4, 5" represent series of concentrations of GP (0, 5, 2.5, 1.25, 0.625, 0.313 mg mL−1), Ce6 (0, 100, 50, 25, 12.5, 6.25 μgmL−1), SiNPs and GP-Ce6-SiNPs (0, 10, 5, 2.5, 1.25, 0.625 mg mL−1). All error bars represent the standard deviation determined from three independent assays. Scale bar: 50 μm.



c) Histological evaluation of different organs (heart, liver, spleen, lung, and kidney) from healthy mice, which are suffered from 10-day treatment of PBS, PBS + irradiation, GP-Ce6-SiNPs, GP-Ce6-SiNPs + irradiation, respectively. Scale bar: 100 μm. d) Ex vivo dual-emission imaging of organs resected from healthy mice after 24 h post injection of GP-Ce6- SiNPs. First row (from left to right): heart (He), liver (Li); second row (from left to right): spleen (Sp), lung (Lu), and kidney (Ki).

Discussion

green fluorescent emission from SiNPs under 405-nm UV excitation is not desirable for in vivo imaging due to its relatively poor penetration depth

→ Ce6 loaded on SiNPs
PDT agent
imaging agent
provide stable red fluorescence signal
improvement of the penetration depth.

two prerequisites for nanoagents
- glucosyl residues
- small size of nanoagents

→ GP-Ce6-SiNPs

minimum number of bacteria discriminated in vivo by nanoagents is 100,000 CFU

→ 100,000 CFU of bacteria are too few to establish a stable infection in immune competent mice

nanoagents

- afford a long-term imaging (4 days)
- exhibiting stable green and red fluorescence signals

nanoagents amount used in our case is relatively high (10 mg mL−1)

- providing strong fluorescence signals even at ultralow concentrations of bacteria
- Achieving desirable treatment effects within a relatively short irradiation time


Thanks!