

Mannose-guided carrier-free nanodrugs for cascade targeted eradication of intracellular bacterial infections

Runyu Han^a, Ya-nan Fu^b, Yuecong Guo^c, Yizhen You^a, Jiali Yang^a, Yaling Wang^c,
Wensheng Xie^{a,*}, Guofeng Li^{a,*}, Xing Wang^{a,*}

^a State Key Laboratory of Organic-Inorganic Composites, Beijing Laboratory of Biomedical Materials, College of Life Science and Technology, Beijing University of Chemical Technology, Beijing 100029, China

^b School of Nanoscience and Engineering, University of Chinese Academy of Sciences, Beijing 101408, China

^c CAS Key Laboratory for Biomedical Effects of Nanomaterials and Nanosafety, CAS Center for Excellence in Nanoscience, National Center for Nanoscience and Technology of China, Beijing 100090, China

ARTICLE INFO

Keywords:

Carrier-free nanodrug
Intracellular bacterial infection
Cascade-targeted delivery
Synchrotron X-ray imaging

ABSTRACT

Intracellular pathogens persist because they hide within host cells, shielding themselves from immune surveillance and limiting antibiotic exposure. Herein, a mannose-functionalized, carrier-free nanodrug (MTBN) is reported that executes a cascade-targeting strategy against intracellular *Staphylococcus aureus* (*S. aureus*). MTBN is generated through a dynamic Schiff-base-driven co-assembly of tobramycin (Tob) and borneol 4-formylbenzoate (BF), followed by mannose conjugation, affording high drug loading, minimal structural complexity, and long-term colloidal stability. Upon administration, MTBN preferentially homes to infected macrophages via mannose-receptor-mediated endocytosis and subsequently escapes lysosomes after acid-triggered de-mannosylation. In the mildly acidic, infection-associated microenvironment, the nanodrug disassembles into smaller fragments that are readily internalized by *S. aureus*, boosting intrabacterial drug accumulation. Cleavage of the Schiff-base linkages then releases Tob and BF in a synchronized manner, producing synergistic bactericidal activity that efficiently eradicates intracellular bacteria. MTBN achieved 99.99% and 99.9% intracellular bacterial killing *in vitro* and *in vivo*, respectively, with efficacy superior to that of free drugs, the physical mixture, and non-targeted TBN. This carrier-free platform provides a promising therapeutic strategy for the treatment of refractory intracellular infections.

Introduction

Intracellular parasitic bacteria are major drivers of chronic infectious diseases, which are often marked by recurrent episodes and difficulty in achieving complete eradication, thereby posing a persistent threat to human health [1–3]. A key pathogenic feature is their ability to invade eukaryotic host cells, which enables escape from humoral immune surveillance and subsequent clearance [4–6]. Once internalized, host cells provide a protected niche that supports bacterial persistence, latency, and relapse, making sterilizing cure challenging [7]. For example, after uptake by macrophages, *Staphylococcus aureus* (*S. aureus*) can adopt a quiescent phenotype that resists lysosomal degradation and perturbs host immune functions, ultimately establishing a stable intracellular reservoir [8–10]. Such intracellular *S. aureus* is associated with severe clinical manifestations, including osteomyelitis, sepsis, and necrotizing

pneumonia [11–13]. However, many conventional antibiotics, including β -lactams and aminoglycosides, penetrate macrophage membranes inefficiently and therefore fail to reach therapeutic intracellular concentrations [2,14,15]. Consequently, improving intracellular antibiotic delivery to achieve effective clearance of intracellular bacteria remains a critical challenge in contemporary anti-infective therapy [16].

The utilization of drug-delivery systems (DDS), particularly nano-carriers such as liposomes, polymers, and metal-organic frameworks, offers substantial promise for treating intracellular bacterial infections [17–21]. By rationally tuning particle size, surface charge, and stimulus-responsive behavior, these platforms can be engineered to promote preferential accumulation in infected cells and to enable controlled, on-demand antibiotic release [16,22,23]. As a result, nanocarrier-based delivery can increase intracellular antibiotic exposure and improve antibacterial efficacy, helping to address the poor

* Corresponding authors.

E-mail addresses: xws@mail.buct.edu.cn (W. Xie), ligf@mail.buct.edu.cn (G. Li), wangxing@mail.buct.edu.cn (X. Wang).

<https://doi.org/10.1016/j.nantod.2026.103120>

Received 21 April 2026; Received in revised form 22 June 2026; Accepted 28 June 2026

Available online 1 July 2026

1748-0132/© 2026 Elsevier Ltd. All rights are reserved, including those for text and data mining, AI training, and similar technologies.

intracellular performance of many conventional agents [24–26]. Despite these advances, several barriers still limit broader translation. Many systems suffer from low drug loading, which restricts achievable intracellular doses, while material-associated toxicity and unintended immune activation can compromise biocompatibility [27–30]. In addition, complicated formulations and manufacturing workflows often impede scalable production and clinical implementation [31–33]. Therefore, there remains a strong need for innovative DDS that combine high loading efficiency, favorable biocompatibility, and simplified design and fabrication to effectively combat intracellular bacterial infections.

Carrier-free nanodrugs represent an emerging class of nano-drug delivery systems in which multiple therapeutic agents or bioactive components self-assemble into nanoscale formulations with minimal or no inert excipients [34–36]. Owing to their carrier-free architecture, these systems not only achieve exceptionally high drug-loading capacity while avoiding carrier-associated toxicity, but also enable intrinsic

combination therapy: the co-assembled components act cooperatively to generate therapeutic outcomes that can surpass the sum of individual effects [37–39]. Accordingly, carrier-free nanodrugs have attracted increasing interest for cancer therapy and for infections caused by drug-resistant bacteria [40–42]. For example, Hu et al. reported a self-assembled PMBA₄ nanodrug composed of polymyxin B and (E)-2-heptenal, which reduced the minimum inhibitory concentration against multidrug-resistant *Pseudomonas aeruginosa* by 100-fold and effectively disrupted bacterial biofilms [43]. Despite these advances, the use of carrier-free nanodrugs for intracellular bacterial infections remains largely unexplored. Most reported systems rely primarily on passive accumulation via the enhanced permeability and retention (EPR) effect and therefore lack active, cell-specific delivery to infected host cells [44]. As a consequence, their targeting efficiency is often insufficient for intracellular infections, and efficient translocation across host cell membranes with controlled drug release at pathogen-relevant

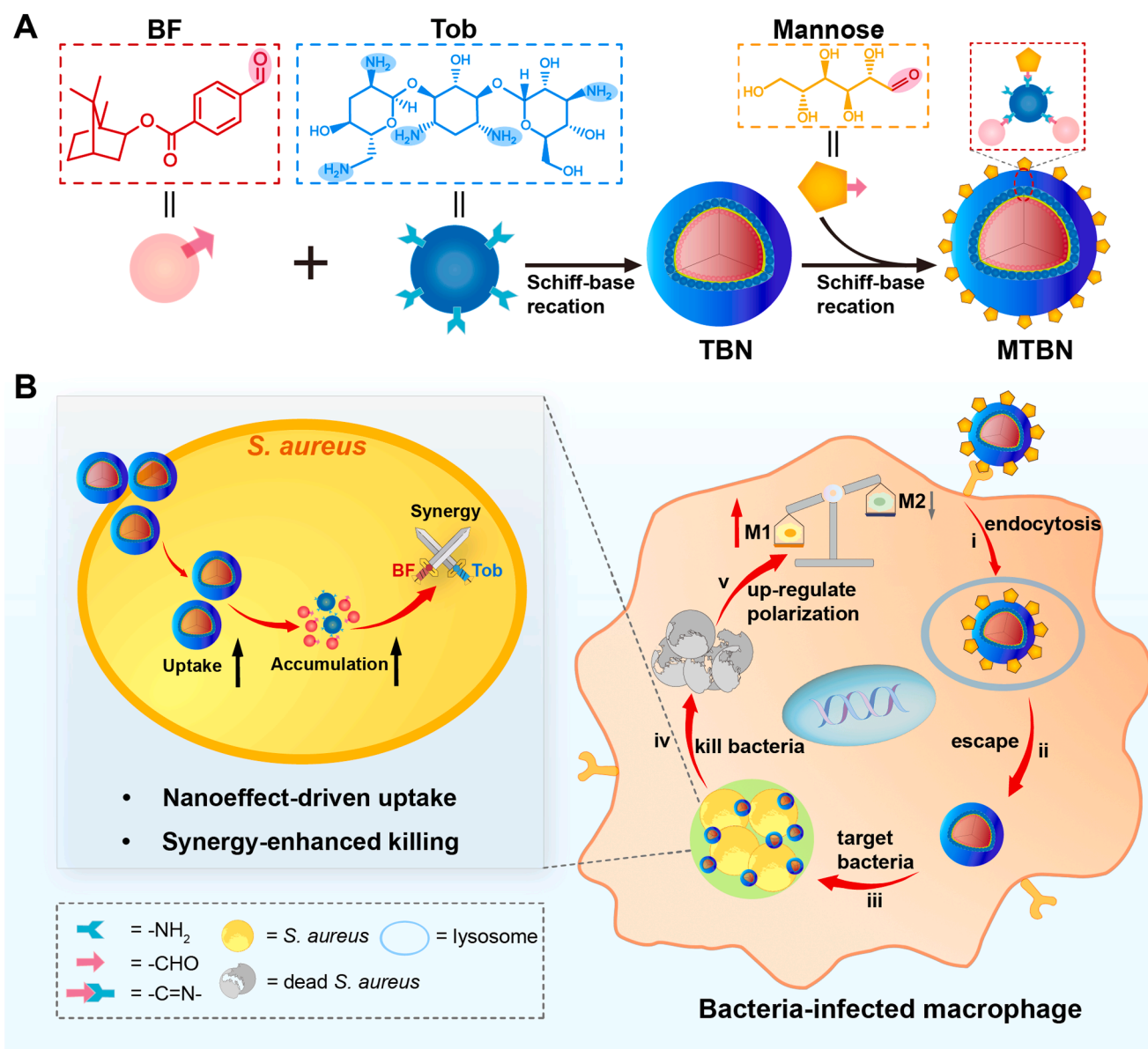


Fig. 1. (A) Schematic illustration of MTBN synthesis. (B) Mechanism of MTBN for treating intracellular bacterial infection: i) MTBN enters cells via mannose receptor-mediated endocytosis. ii) Mannose moieties are removed in lysosomes, allowing MTBN to escape into the cytosol. iii) MTBN targets intracellular bacteria. iv) In the weakly acidic microenvironment at the infected site, TBN disassembles into smaller nanofragments that are readily internalized by bacteria, resulting in enhanced intrabacterial drug accumulation. Subsequent cleavage of Schiff-base bonds enables BF and Tob to act synergistically to disrupt bacterial membranes and efficiently kill intracellular bacteria. v) Upregulation of M1/M2 polarization.

subcellular sites remains challenging, ultimately limiting therapeutic efficacy.

To address the above limitations, tobramycin (Tob) and borneol 4-formylbenzoate (BF) are first co-assembled via dynamic Schiff base chemistry to form carrier-free nanodrugs (TBN), while a fraction of unreacted primary amines remains exposed on the particle surface [39]. These residual amines subsequently serve as reactive handles for mannose conjugation through Schiff base formation, yielding actively targeted MTBN (Fig. 1). The mannose decoration is designed to promote selective uptake by infected macrophages via specific recognition of the CD206 receptor expressed on their surface [45–47]. After internalization, mannose moieties are cleaved in the acidic lysosomal milieu, exposing positively charged amino groups that facilitate lysosomal escape into the cytosol [48,49]. In the weakly acidic microenvironment associated with intracellular infection, TBN further disassembles into smaller nanofragments, which are more readily internalized by *S. aureus* and thereby enhance intrabacterial drug accumulation. Subsequent cleavage of the Schiff base linkages allows Tob and BF to act cooperatively and exert synergistic antibacterial activity. In parallel, MTBN enhances antibacterial efficacy by modulating innate immune responses and shifting macrophage polarization toward a pro-inflammatory phenotype. Collectively, the combination of efficient intracellular delivery, infection-responsive disassembly, synergistic antibacterial action, and immunomodulation enables MTBN to function as a cascade-targeted therapeutic strategy for refractory intracellular bacterial infections.

Results and discussion

Synthesis and characterization of MTBN

The preparation of MTBN is schematically depicted in Fig. 1A. Briefly, hydrophilic Tob is anchored at the dichloromethane/water emulsion interface through association with hydrophobic BF, leading to the formation of carrier-free TBN with a hydrophilic surface. Benefiting from this oil-water interfacial templating effect, Tob is enriched on the outer layer, thereby retaining a fraction of accessible amine groups. Mannose is then conjugated to these surface amines via dynamic Schiff base chemistry to yield mannose-functionalized MTBN. Scanning electron microscopy (SEM) images show that both TBN and MTBN are uniformly spherical, with particle sizes of 100–400 nm (Fig. 2A). Transmission electron microscopy (TEM) further reveals a solid interior for TBN, whereas MTBN displays a distinct core-shell architecture, consistent with surface mannosylation (Fig. 2B). Dynamic light scattering (DLS) measurements indicate an increase in hydrodynamic diameter from 191 nm (TBN) to 232 nm (MTBN), and a concomitant decrease in zeta potential from +42.9 mV to +32.5 mV, confirming successful conjugation (Fig. 2C, D). In addition, MTBN remains colloidally stable in phosphate-buffered saline (PBS) and fetal bovine serum (FBS) for at least 7 days, maintaining a diameter below 300 nm and a polydispersity index (PDI) consistently below 0.25 (Fig. 2E, F).

X-ray photoelectron spectroscopy (XPS) was used to probe the surface chemistry of MTBN and to verify mannose installation. Compared with TBN, MTBN exhibits a pronounced increase in the C3 (C=N)

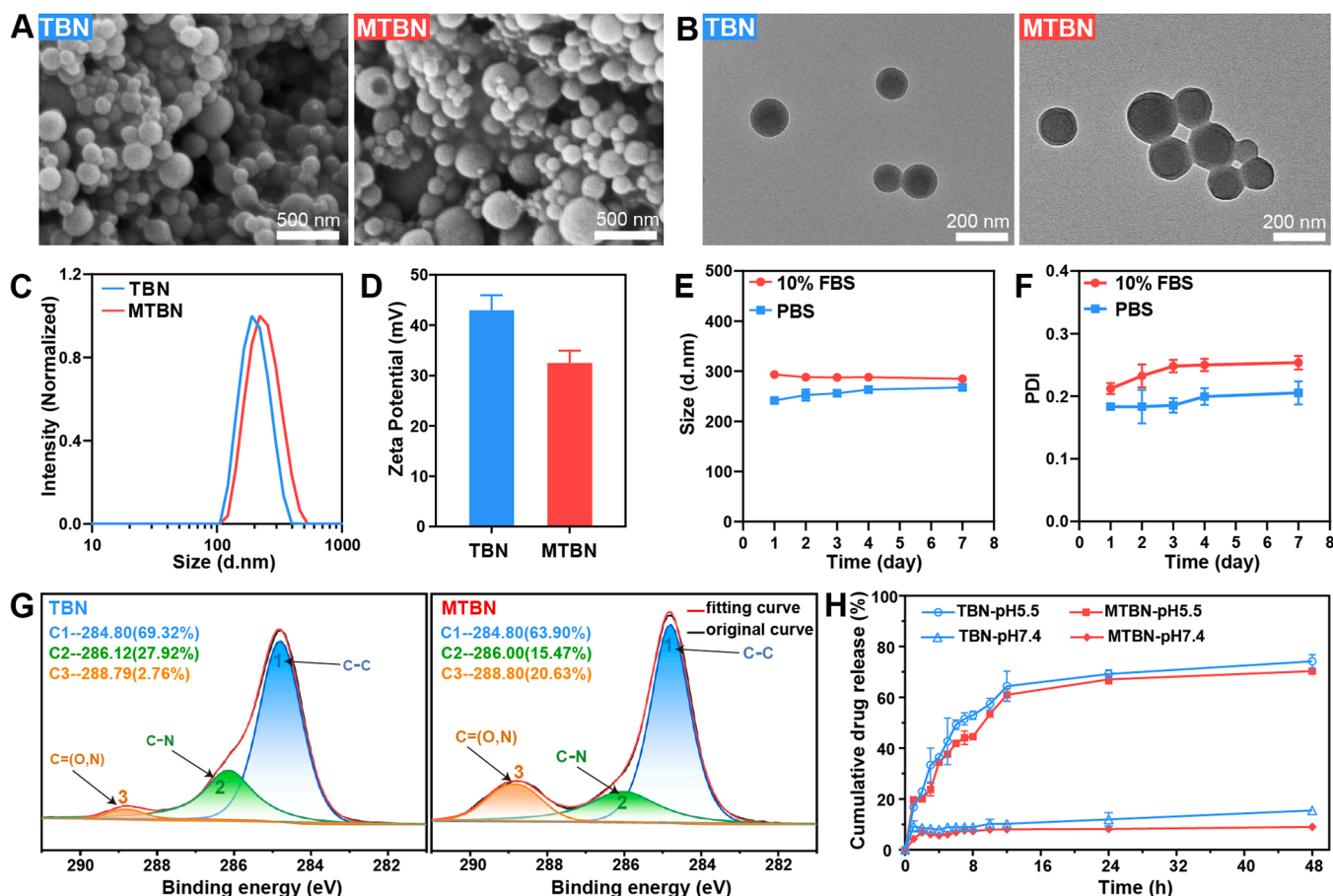


Fig. 2. Characterization of MTBN nanodrugs. (A) SEM images of TBN and MTBN. (B) TEM images of TBN and MTBN. (C) Hydrodynamic size distributions of TBN and MTBN measured by DLS ($n = 3$). (D) Zeta potential measurements of TBN and MTBN ($n = 3$). (E) Time-dependent size changes of TBN and MTBN in different culture media ($n = 3$). (F) Time-dependent PDI variations of TBN and MTBN in different culture media ($n = 3$). (G) XPS C1s spectra of TBN and MTBN. (H) pH-responsive release profiles of tobramycin from TBN and MTBN under various pH conditions ($n = 3$).

component together with a concomitant decrease in the C2 (C-N) component in the C1s spectrum, which is attributed to the higher abundance of Schiff base linkages introduced during mannose conjugation (Fig. 2G). Consistent evidence is provided by ^1H NMR spectroscopy (Fig. S2). The characteristic -CHO resonance at δ 10.1 ppm disappears in TBN, while new signals assigned to Schiff base formation emerge at δ 8.35–8.65 ppm, indicating successful conjugation of Tob and BF into TBN. After mannose grafting, MTBN shows an additional Schiff base resonance at δ 8.52 ppm, accompanied by distinct mannose-derived hydroxyl proton signals at δ 4.07–4.82 ppm. Further support is obtained from Fourier-transform infrared (FTIR) spectroscopy (Fig. S3), where the C=N stretching band at 1600 cm^{-1} is markedly enhanced in MTBN relative to TBN. Meanwhile, intensified O-H stretching (3421 cm^{-1}) and C-H vibration (1435 cm^{-1}) bands are observed, consistent with the introduction of mannose-associated functional groups. Collectively, these spectroscopic results confirm that mannose is covalently anchored to the TBN surface through Schiff base bonds.

The drug loading of Tob in MTBN is determined to be 38.47% using the o-phthalaldehyde method [50], whereas the mannose content is quantified as 15.66% by the phenol-sulfuric acid assay [51]. Based on these measurements, the BF loading is calculated to be 45.87% (Table S1). From the loading contents, the molar composition of MTBN

is further derived, giving a Tob: BF molar ratio of 1:2 and a Tob: mannose molar ratio of 1:1, which is consistent with the proposed core-shell structural model. The pH-responsive release behavior of TBN and MTBN is then evaluated under physiological (PBS, pH 7.4) and mildly acidic (PBS, pH 5.5) conditions. Under pH 5.5, Tob is released substantially faster, with 49% cumulatively released from MTBN within 6 h, compared with only 9% at pH 7.4 over the same period. After 48 h, cumulative Tob release reaches 74% at pH 5.5 but increases only to 15% at pH 7.4. Notably, MTBN exhibits a release profile comparable to that of TBN, indicating that mannose modification does not measurably alter Tob release and that both nanodrugs retain acid-responsive release behavior (Fig. 2H). Additionally, MTBN was pretreated in PBS (pH 5.5) for different durations and then examined by SEM to visualize its acid-triggered structural evolution. As shown in Fig. S6, approximately half of the MTBN assemblies disintegrate into $\leq 100\text{ nm}$ fragments by 6 h, and this disassembly becomes more pronounced at 9 h. By 12 h, the larger spherical architectures are no longer observed, leaving predominantly $\leq 100\text{ nm}$ fragments. Collectively, these time-dependent morphological changes suggest that the mildly acidic microenvironment promotes cleavage of the Schiff base linkages, which drives MTBN disassembly into smaller structures and enables acid-responsive drug release.

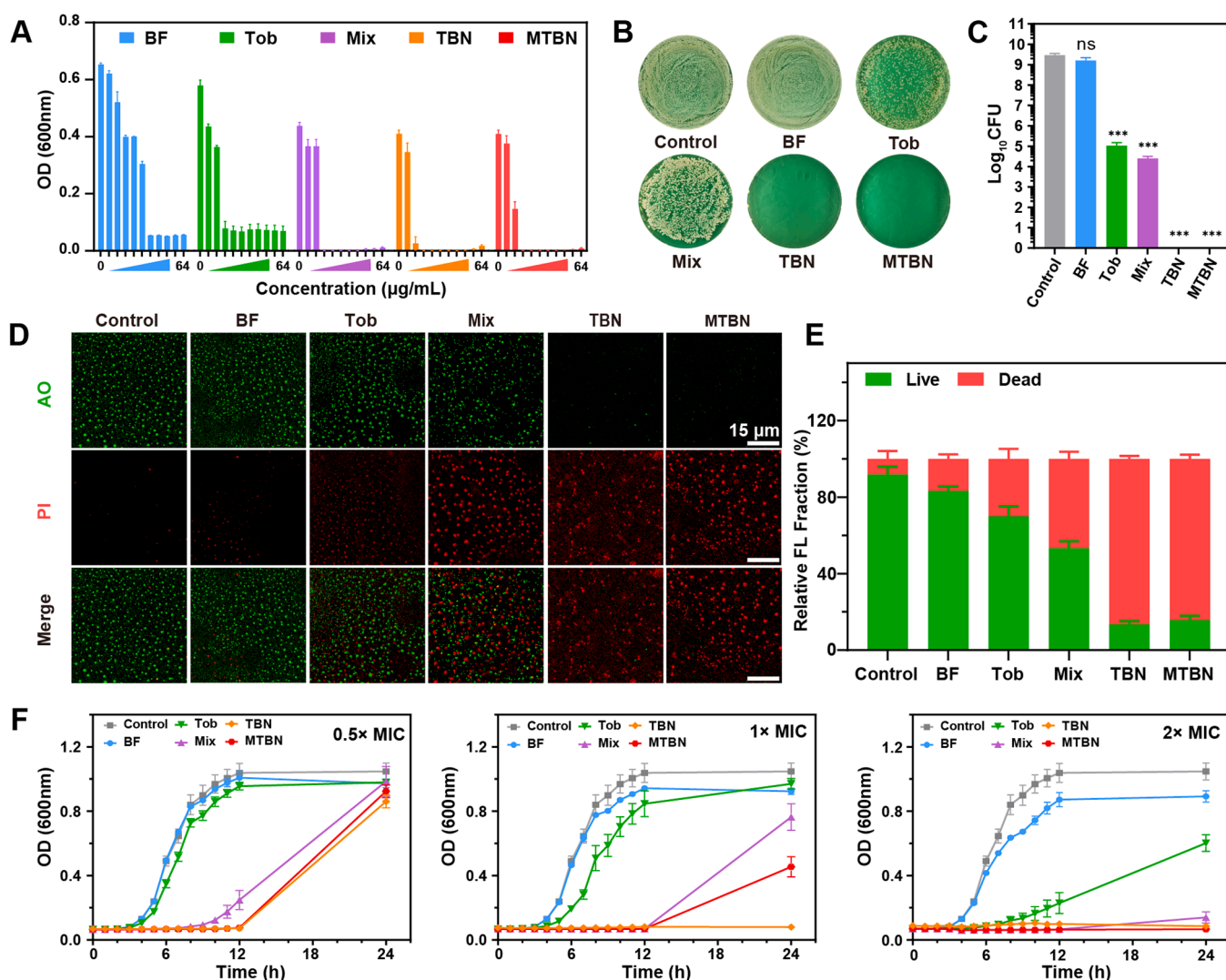


Fig. 3. Evaluation of the extracellular antibacterial activity of MTBN. (A) MICs of BF, Tob, Mix, TBN, and MTBN against *S. aureus* ($n = 3$). (B) Representative colony images and (C) quantitative CFU analysis of extracellular antibacterial assays after treatment with BF, Tob, Mix, TBN, and MTBN ($n = 3$). (D) Live/dead staining images of *S. aureus* following different treatments. (E) ImageJ quantification of live/dead fluorescence fractions ($n = 3$). (F) Growth curves of *S. aureus* treated with BF, Tob, Mix, TBN, and MTBN at $0.5 \times \text{MIC}$, $1 \times \text{MIC}$, and $2 \times \text{MIC}$ ($n = 3$).

Extracellular antibacterial activity

To evaluate extracellular antibacterial activity against *S. aureus*, the minimum inhibitory concentrations (MICs) of BF, Tob, their physical mixture (Mix), TBN, and MTBN were determined. As shown in Fig. 3A, the MICs are 4 µg/mL for BF, 0.5 µg/mL for Tob, 0.5 µg/mL for Mix, 0.25 µg/mL for TBN, and 0.5 µg/mL for MTBN. TBN exhibits the lowest MIC, indicating enhanced inhibitory potency relative to the single drugs and Mix. Notably, although the MIC of MTBN matches that of Mix, measurable growth inhibition is already observed at 0.25 µg/mL, whereas Mix does not exhibit significant antibacterial activity at the same concentration. The minimum bactericidal concentrations (MBCs) further support this trend. BF, Tob, and Mix display MBCs of 8, 2, and 2 µg/mL, respectively, whereas both TBN and MTBN achieve an MBC of 1 µg/mL (Fig. S7). Consistent with these results, colony-counting assays performed after incubating *S. aureus* with BF, Tob, Mix, TBN, or MTBN at an identical dose concentration (1 µg/mL) show that BF produces no statistically significant reduction in colony-forming unit (CFU) relative to the control, while Tob and Mix reduce bacterial burdens by 4.4 and 5.0 log₁₀ CFU, respectively (Fig. 3B, C). In contrast, no viable colonies are detected after treatment with either TBN or MTBN, demonstrating complete bacterial eradication at this dose. Collectively, these findings indicate that the assembled carrier-free nanodrugs display potent extracellular antibacterial activity against planktonic *S. aureus*. Live/Dead staining further corroborates these quantitative results, both nanodrugs induce predominant red fluorescence with an almost complete loss of green signals (Fig. 3D, E), indicative of severe membrane damage and near-total loss of viability [52]. By contrast, BF retains predominantly green fluorescence similar to the control, whereas Tob and Mix induce only a modest increase in red signals, consistent with their limited bactericidal activity.

Systematic assessment of antibacterial kinetics was further conducted by monitoring *S. aureus* growth at three dose levels ($0.5 \times \text{MIC}$, $1 \times \text{MIC}$, and $2 \times \text{MIC}$), with BF, Tob, Mix, TBN, and MTBN evaluated in parallel (Fig. 3F). At $0.5 \times \text{MIC}$, neither Tob nor BF produces appreciable growth inhibition, whereas Mix delays the onset of the logarithmic phase from 4 h to 8 h. A more pronounced delay is observed for both TBN and MTBN, with the exponential growth phase postponed to 12 h. When the dose is increased to $1 \times \text{MIC}$, TBN completely suppresses bacterial proliferation over the monitored period, while MTBN maintains sustained growth inhibition; by contrast, the Mix group exhibits regrowth after 12 h, indicating limited durability. At $2 \times \text{MIC}$, MTBN, TBN, and Mix all display strong antibacterial activity, whereas Tob and BF only transiently inhibit growth at early time points, followed by gradual regrowth after 12 h. Overall, TBN and MTBN consistently outperform the single-drug formulations and their physical mixture, achieving substantial growth delay even at reduced concentrations.

Antibacterial mechanism of MTBN

To assess the ability of MTBN to target planktonic bacteria, GFP-labeled *S. aureus* (GFP-*S. aureus*) is co-incubated with Nile Red-labeled MTBN (NR@MTBN) and analyzed by confocal laser scanning microscopy (CLSM). CLSM images show pronounced overlap between the green (GFP-*S. aureus*) and red (NR@MTBN) signals, indicating strong colocalization (Fig. S8A). This spatial correspondence is further quantified using ImageJ, where the red fluorescence profile of NR@MTBN closely matches the green fluorescence profile of GFP-*S. aureus* (Fig. S8B), yielding Pearson's correlation coefficient (PCC) above 0.6 (Fig. S8C). These results support efficient bacterial association of MTBN. Consistent with this observation, zeta potential measurements reveal that the bacterial surface potential increases markedly after incubation with positively charged MTBN, due to electrostatic interactions with negatively charged phosphate ester groups on the bacterial cell wall (Fig. S9). MTBN adsorbs onto the bacterial surface through electrostatic attraction, thereby promoting bacterial accumulation and perturbing

bacterial surface properties [53]. After MTBN is shown to efficiently associate with bacterial surfaces, synchrotron X-ray imaging is further applied to resolve its spatial distribution and internalization behavior at the bacterial scale. TBN is first labeled with Tetra(4-iodophenyl) ethylene to afford iodine-tagged nanodrugs (I@TBN), and successful iodine incorporation is verified by DLS and energy-dispersive X-ray spectroscopy (EDS) (Figs. S10 and S11). I@TBN is then incubated with *S. aureus* prior to synchrotron imaging. In the two-dimensional cross-sectional images (Fig. 4A), bacteria in the control group show relatively higher X-ray absorption at the cell envelope and lower absorption in the cytoplasmic region. By contrast, the I@TBN-treated group exhibits markedly enhanced absorption signals within the bacterial interior, suggesting that I@TBN crosses the cell-wall barrier and accumulates intra bacterially. This inference is further strengthened by three-dimensional reconstructions, which reveal continuously distributed iodine signals throughout the bacterial volume in the treated group (Fig. 4B, Video S1). Consistently, quantitative analysis of the linear absorption coefficient (LAC) shows significantly higher LAC values in the I@TBN-treated group than in the control (Fig. 4C), providing quantitative support for intrabacterial enrichment and effective internalization of the iodinated nanodrugs.

To clarify the synergistic antibacterial interactions among the MTBN components, the combined activity of BF and Tob was first evaluated using a checkerboard assay. As shown in Fig. 4D, fractional inhibitory concentration index (FICI) of 0.5 was obtained when BF and Tob were applied at 1 µg/mL and 0.25 µg/mL, respectively, confirming synergistic interaction [54]. The contribution of mannose to Tob-mediated antibacterial efficacy was then examined [55]. As shown in Fig. 4E and Fig. S12, increasing the mannose concentration to ≥ 16 mM reduced the *S. aureus* survival rate from 55% to 30% relative to mannose-free conditions, indicating that mannose markedly potentiated Tob activity. Exogenous carbohydrates and metabolites have been reported to enhance aminoglycoside activity by promoting bacterial metabolism and antibiotic uptake [55–57]. Therefore, the mannose-enhanced Tob activity may be partially attributed to metabolite-assisted sensitization. Collectively, these results suggest that the MTBN constituents' function cooperatively to achieve enhanced antibacterial performance.

To determine whether such cooperation translated into structural damage, bacterial morphology after each treatment was examined by SEM. PBS-treated *S. aureus* maintained intact cell morphology with clear boundaries, whereas only minor surface deformation was observed after exposure to BF or Tob. In the Mix group, moderate membrane disruption was detected. By contrast, treatment with TBN or MTBN caused severe structural collapse accompanied by evident cytoplasmic leakage, consistent with pronounced bactericidal activity (Fig. 4F). Membrane depolarization was further assessed using the potential-sensitive probe 3,3'-Diethyloxycarbocyanine (DiOC₂(3)) [58]. Both TBN and MTBN induced substantial depolarization, and the effect was markedly stronger than that produced by the single-drug and Mix groups (Fig. 4G). Consistently, ortho-Nitrophenyl-β-galactoside (ONPG) assays showed that TBN and MTBN significantly increased membrane permeability (Fig. 4H). [59] Protein leakage measurements provided additional support: the BCA assay revealed significantly elevated extracellular protein levels in the TBN- and MTBN-treated groups, exceeding those observed in the Mix group (Fig. 4I).

In summary, MTBN kills *S. aureus* via a cascade mechanism in which electrostatic adsorption promotes bacterial enrichment, disassembly under mildly acidic infection microenvironment enables bacterial internalization and elevates intrabacterial drug levels, and subsequent Schiff-base cleavage allows Tob and BF to act synergistically, ultimately causing severe membrane dysfunction and bacterial death.

Evaluation of mannose-mediated cellular uptake and lysosomal escape

Mannose is grafted onto the MTBN surface to promote targeted

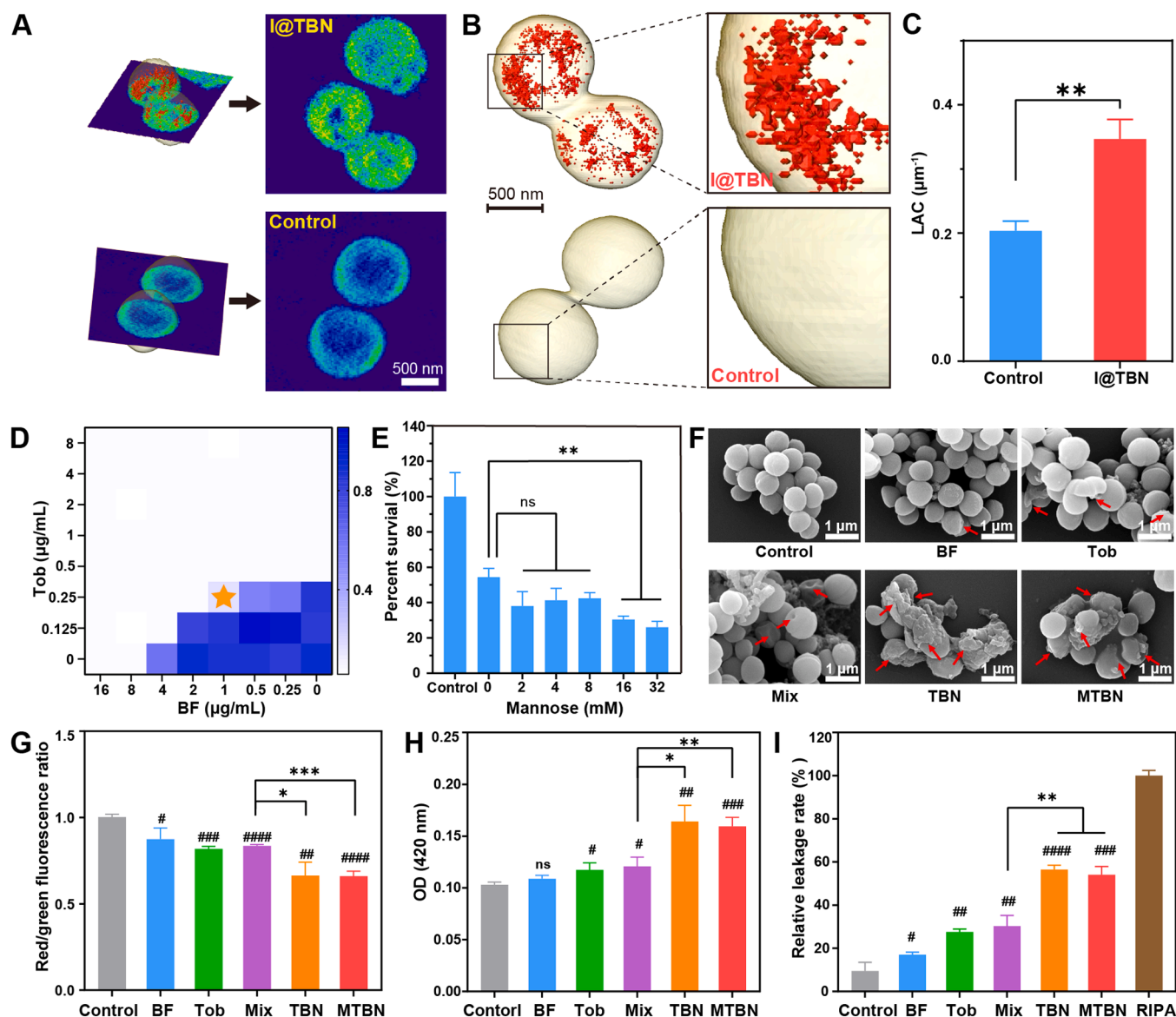


Fig. 4. Antibacterial mechanism of MTBN. (A) Synchrotron radiation imaging cross-sectional images of the I@TBN group and the control group. (B) 3D reconstruction of synchrotron radiation imaging for the I@TBN group and the control group. The red signal corresponds to the iodine elemental signal from iodine-labeled nanodrugs. (C) Statistical comparison of LAC between the I@TBN group and the control group ($n = 3$). (D) Checkerboard assay evaluating the synergistic antibacterial effects of BF and Tob. (E) Mannose-enhanced antibacterial activity of Tob. (F) SEM images of *S. aureus* after treatment with different formulations, with red arrows indicating cell wall and membrane shrinkage or disruption. (G) Bacterial membrane depolarization after treatment with different formulations ($n = 3$). (H) Changes in bacterial membrane permeability after treatment with different formulations ($n = 3$). (I) Protein leakage from *S. aureus* after treatment with different formulations ($n = 3$). # indicates statistical significance between the experimental group and the control group.

delivery toward infected macrophages. To examine whether mannose functionalization improves cellular uptake, coumarin-6 (C6) is loaded as a fluorescent tracer to generate C6@TBN and C6@MTBN, and time-dependent internalization in RAW264.7 cells is monitored by CLSM (Fig. 5A, B). C6@MTBN shows strong intracellular accumulation as early as 2 h, followed by a further increase at 4 and 6 h, after which the signal approaches a plateau. In contrast, C6@TBN exhibits consistently weaker intracellular fluorescence, the signal stabilizes after 6 h, and remains markedly lower than that of C6@MTBN even at 12 h. These observations indicate that mannose decoration substantially enhances cellular internalization of the nanodrugs. To verify receptor involvement, a competitive inhibition assay is performed using free mannose as a blocker of mannose receptors [26]. RAW264.7 cells are pre-incubated with free mannose for 1 h and then treated with C6@MTBN or C6@TBN. Both CLSM imaging and flow cytometry show that receptor blocking

significantly reduces C6@MTBN uptake, while exerting little effect on C6@TBN internalization (Fig. 5C, D). Together, these results support that C6@MTBN enters macrophages predominantly through mannose receptor-mediated endocytosis.

Macrophages typically internalize nanodrugs through endocytosis and traffic them to lysosomes, where premature degradation can markedly reduce cytosolic drug availability and compromise antibacterial efficacy against intracellular pathogens [48,49]. Therefore, promoting lysosomal escape is essential for maximizing intracellular bioavailability. To visualize the lysosomal trafficking and escape behavior of MTBN, the nanodrug is labeled with the AIE fluorophore TPE-CHO to generate AIE@MTBN (Ex/Em = 369/488 nm, Fig. S14A) [60,61]. Notably, AIE@MTBN exhibits pH-dependent fluorescence attenuation: its emission intensity gradually decreases as the pH is lowered from 7.4 to 3.0, consistent with acid-triggered nanodrug

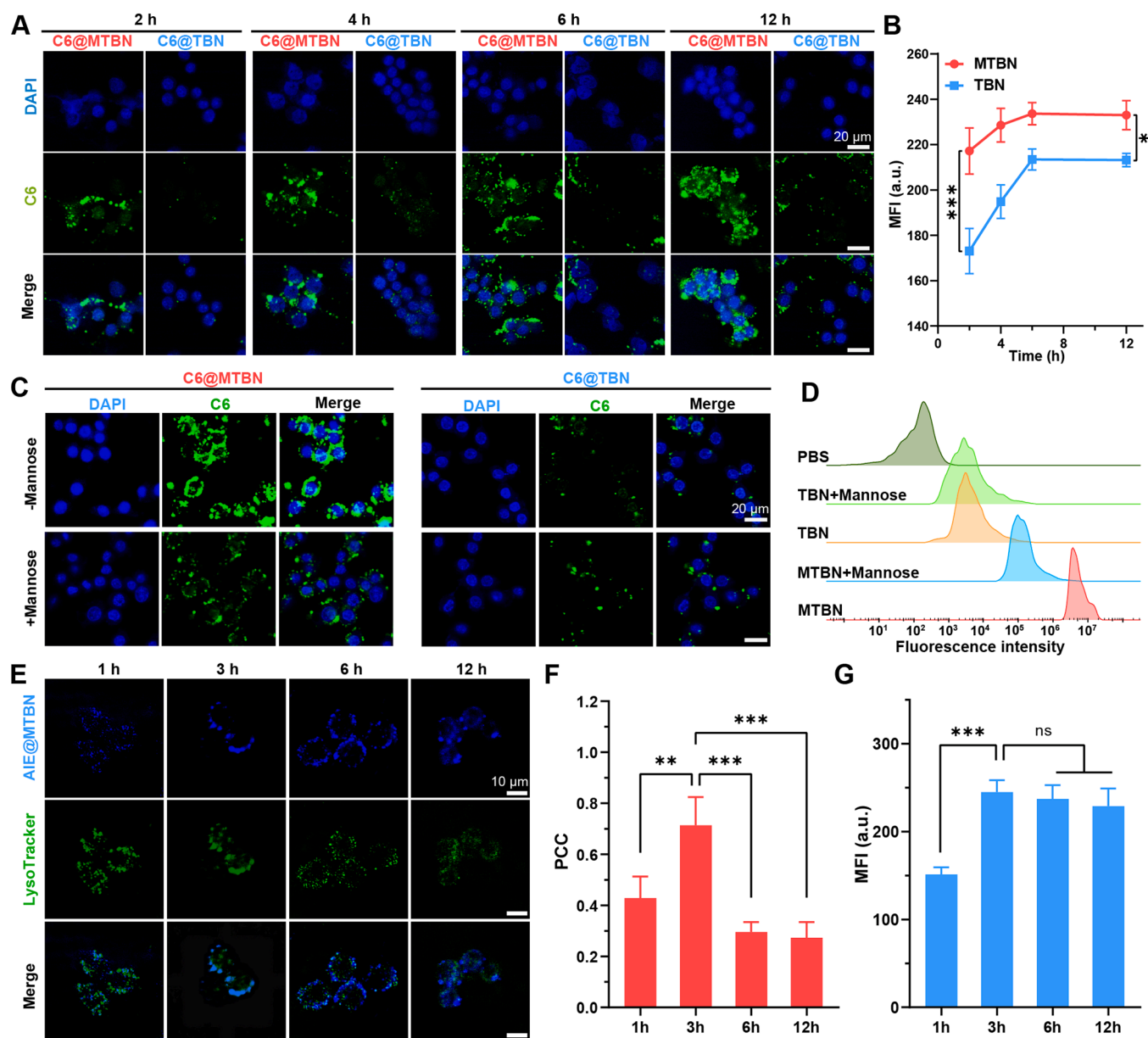


Fig. 5. Mannose-mediated endocytosis and lysosomal escape of MTBN. (A) CLSM images of RAW264.7 macrophages incubated with C6@MTBN or C6@TBN for 2, 4, 6, and 12 h, with nuclei stained by DAPI. (B) Quantitative analysis of cellular uptake based on mean fluorescence intensity (MFI) using ImageJ (n = 3). (C) CLSM images of RAW264.7 macrophages incubated with C6@MTBN or C6@TBN for 3 h in the presence of competitive inhibitor mannose. (D) Flow cytometric analysis of C6 fluorescence intensity in RAW264.7 macrophages. (E) CLSM images showing the colocalization of AIE@MTBN with lysosomes in RAW264.7 macrophages after 1, 3, 6, and 12 h of incubation, with lysosomes stained by LysoTracker Green. (F) PCC between AIE@MTBN and lysosomes analyzed by ImageJ (n = 3). (G) Quantitative analysis of AIE@MTBN fluorescence intensity in RAW264.7 macrophages using ImageJ (n = 3).

disassembly (Fig. S14B), and this trend is also evident under 365 nm UV illumination (Fig. S14C). This pH sensitivity enables AIE@MTBN to serve as fluorescent reporter for tracking nanodrug behavior within acidic organelles. Lysosomal escape is then assessed by CLSM using lysosomal markers. At 3 h, AIE@MTBN is largely confined to lysosomes, showing strong colocalization with a PCC of 0.7. With prolonged incubation, the blue fluorescence becomes increasingly dispersed throughout the cytoplasm, accompanied by pronounced reduction in lysosomal colocalization, and the PCC drops below 0.3 after 6 h (Fig. 5E, F). In parallel, ImageJ-based quantification shows that the intracellular fluorescence intensity reaches a maximum at 3 h and remains relatively stable thereafter (Fig. 5G), suggesting that AIE@MTBN largely completes cellular entry by 3 h and retains its fluorescence signal during the escape process. Accordingly, these results indicate that MTBN is capable

of effective lysosomal escape.

Intracellular bacterial targeting and antibacterial activity

MTBN was previously shown to exhibit strong association with extracellular *S. aureus*. To determine whether a similar targeting behavior occurs intracellularly, in situ colocalization study is performed in GFP-*S. aureus* infected RAW264.7 macrophages using NR@MTBN. After 24 h of incubation, nuclei are counterstained with DAPI and the cells are analyzed by CLSM. As shown in Fig. 6A, NR@MTBN red fluorescence extensively overlaps with the green GFP-*S. aureus* signal, yielding distinct yellow regions in the merged images and indicating close spatial proximity between MTBN and intracellular bacteria. Z-stack 3D CLSM imaging further visualizes this overlap throughout the

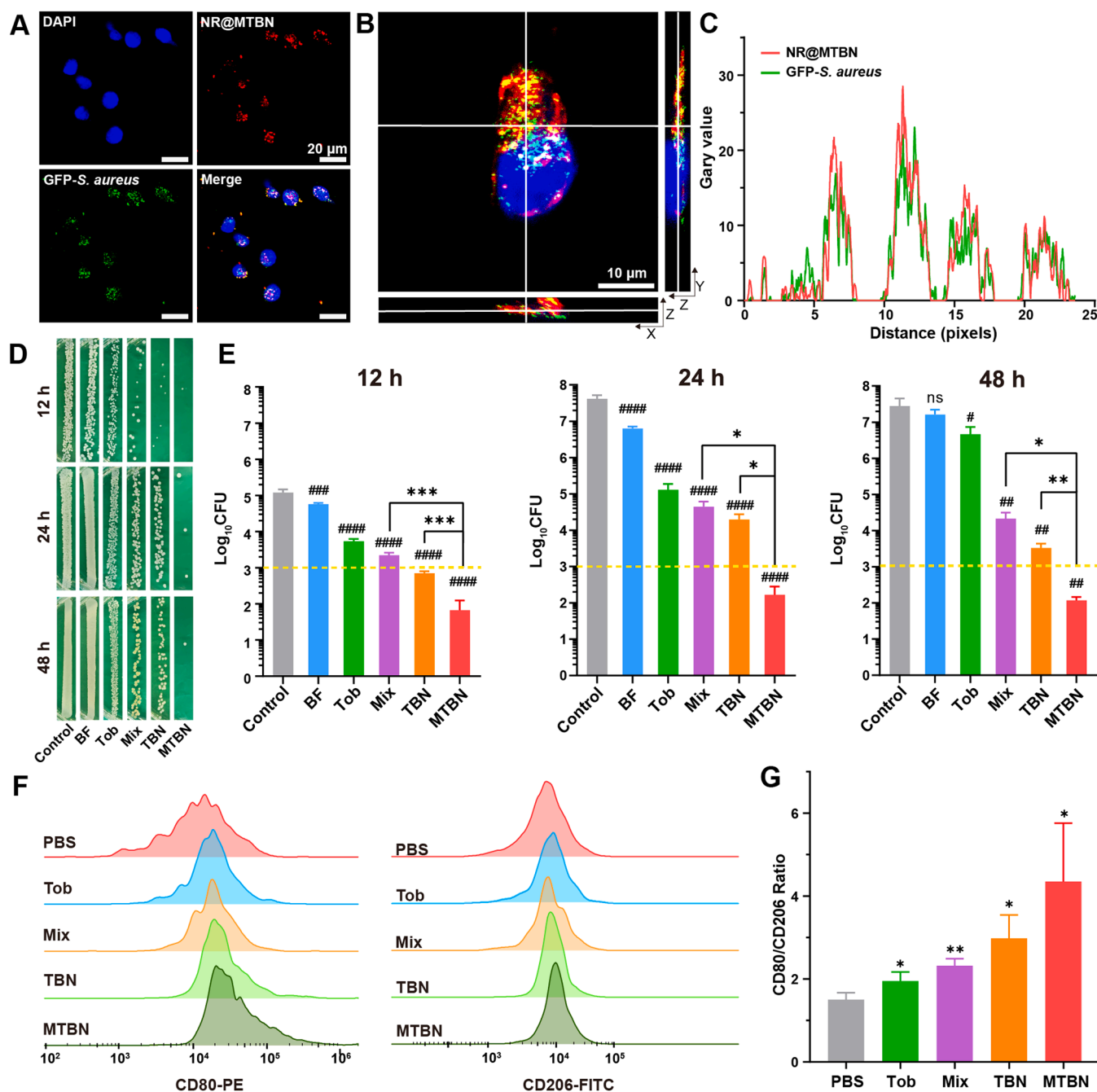


Fig. 6. Intracellular targeting and antibacterial activity of MTBN. (A) CLSM images showing the colocalization of NR@MTBN (red) with intracellular GFP-*S. aureus* (green) in RAW264.7 macrophages after 24 h incubation. (B) Z-stack 3D confocal images illustrating the spatial colocalization between NR@MTBN and intracellular *S. aureus*. (C) Fluorescence intensity distribution profiles of NR@MTBN and GFP-*S. aureus*. (D) Representative colony images of intracellular *S. aureus* after different treatments. (E) Quantitative CFU analysis of intracellular *S. aureus* in RAW264.7 cells ($n = 3$). (F) Flow cytometry analysis of M1 (CD80) and M2 (CD206) markers in infected RAW264.7 macrophages after different treatments. (G) Statistical analysis of the CD80/CD206 expression ratio in macrophages after treatment ($n = 3$). # indicates statistical significance between the experimental group and the control group.

cellular volume, supporting intracellular colocalization (Fig. 6B). Quantitative colocalization metrics revealed substantial spatial overlap between NR@MTBN and intracellular GFP-*S. aureus* (Fig. 6C), as indicated by PCC > 0.6 (Fig. S15). Taken together, MTBN enters macrophages via mannose receptor-mediated endocytosis and, after lysosomal escape, colocalizes with intracellular *S. aureus*, supporting its intracellular antibacterial activity.

The intracellular antibacterial efficacy of MTBN is evaluated in *S. aureus*-infected RAW264.7 macrophages. Infected cells are treated with MTBN (16 μ g/mL), while BF, Tob, Mix, TBN, and PBS serve as

controls, and intracellular bacteria are quantified by colony counting. Across all time points, MTBN achieves the most pronounced intracellular clearance, maintaining viable bacterial levels below 3.0 log₁₀ CFU/mL (Fig. 6D, E). At 12 h, the PBS group remains at 5.0 log₁₀ CFU/mL, with only marginal reductions observed for BF and Tob. Although Mix and TBN reduce intracellular bacteria, MTBN lowers the CFU to < 3.0 log₁₀ CFU/mL and remains significantly more effective than Mix and TBN (Mix vs MTBN, ***, $p = 0.0006$; TBN vs MTBN, ***, $p = 0.0007$). At 24 h, bacterial outgrowth is evident in the PBS group, whereas MTBN continues to suppress intracellular bacteria to

< 3.0 log₁₀ CFU/mL, again outperforming Mix and TBN (Mix vs MTBN, *, $p = 0.0107$; TBN vs MTBN, *, $p = 0.0137$). When the incubation is extended to 48 h, BF no longer differs significantly from PBS (ns, $p = 0.4857$), while MTBN still maintains intracellular bacterial counts below 3.0 log₁₀ CFU/mL and remains significantly lower than Mix and TBN (Mix vs MTBN, *, $p = 0.023$; TBN vs MTBN, **, $p = 0.0059$), indicating sustained intracellular antibacterial activity (99.99% intracellular bacterial killing *in vitro*). This superior performance is attributable to the combined effects of mannose-mediated uptake, lysosomal escape, acid-triggered disassembly/drug release, and cooperative antibacterial action.

Intracellular bacteria are known to dampen pro-inflammatory (M1) macrophage polarization while favoring anti-inflammatory (M2) phenotypes, thereby facilitating immune evasion [62]. To explore whether MTBN contributes to intracellular bacterial clearance by reshaping macrophage phenotype, polarization markers were analyzed by flow cytometry. Relative to PBS-treated controls, CD80 expression was increased after treatment with Tob, Mix, TBN, and MTBN, with the most pronounced upregulation observed for MTBN, whereas CD206 levels showed a modest decrease across all treatment groups (Fig. 6F). Accordingly, the CD80/CD206 ratio was significantly elevated in the MTBN group (Fig. 6G), indicating a shift toward an M1-skewed polarization state that may strengthen macrophage-mediated elimination of intracellular *S. aureus* [63].

In vivo targeting and antibacterial efficacy

A murine thigh muscle infection model is established to evaluate the *in vivo* infection-site targeting of MTBN (Fig. 7A) [64]. Specifically, 100 μ L of *S. aureus* suspension and 100 μ L of PBS are injected into the left and right thigh muscles, respectively, to define infected and contralateral uninfected sites. After 12 h, Cy7-labeled MTBN (Cy7@MTBN) is administered intravenously, and its biodistribution is monitored using an IVIS imaging system. As shown in Fig. 7B and C only weak fluorescence is detected at both thighs at 1 h post-injection. By 3 h, fluorescence at the infected site increases markedly and becomes significantly higher than that at the uninfected site (*, $p = 0.0417$). At 6 h, the signal is mainly observed in the liver and the infected thigh, and it continues to accumulate at the infected site at later time points. At 24 h, the infected site reaches maximal fluorescence intensity, remaining markedly higher than the uninfected site (***, $p = 0.0002$). In contrast, the free Cy7 probe is predominantly cleared through hepatic metabolism with minimal infection-site retention. *Ex vivo* imaging at 24 h further confirms preferential accumulation of Cy7@MTBN in the liver, kidneys, and infected tissue, with fluorescence at the infection site exceeding that of the uninfected site by more than twofold (Fig. 7D, **, $p = 0.0075$). Collectively, these results indicate that MTBN achieves infection-site-selective accumulation and prolonged retention in *S. aureus*-infected tissues.

To assess the *in vivo* antibacterial efficacy of MTBN, a *S. aureus*-induced peritonitis model was established (Fig. 7F). This model is widely used to investigate intracellular bacterial infections and enables discrimination between extracellular and intracellular bacteria [65]. Mice were first infected via intraperitoneal injection of *S. aureus* suspension (1×10^8 CFU/mL). After 24 h, various treatment groups were administered intraperitoneal injections of the respective formulations, and treatment was continued for 24 h. As shown in Fig. 7G, all mice receiving TBN or MTBN survived, whereas survival decreased to 83% in the Mix group and 50% in the PBS group. After 24 h of treatment, peritoneal lavage fluid was collected to quantify total, intracellular, and extracellular bacterial CFU (Fig. 7H, I). Mix did not significantly reduce intracellular *S. aureus* (ns, $p = 0.1577$), while both TBN and MTBN produced pronounced intracellular antibacterial effects. Notably, MTBN achieved significantly greater intracellular bacterial clearance than TBN (**, $p = 0.0095$), reducing CFU levels to below 2.0 log₁₀ CFU/mL (99.9% intracellular bacterial killing *in vivo*). For extracellular bacteria,

both TBN and MTBN also showed strong antibacterial activity, and their clearance was significantly superior to that of Mix (**, $p = 0.0061$), consistent with the targeted delivery and acid-triggered disassembly/drug release of the carrier-free nanodrugs. In addition, serum cytokine analysis indicated that MTBN attenuated infection-associated pro-inflammatory cytokine expression (Fig. S18). This finding reflects effective infection control by MTBN and reduced infection-driven inflammatory stimulation *in vivo*. Collectively, MTBN demonstrated robust *in vivo* efficacy, with enhanced clearance of intracellular *S. aureus* and concomitant mitigation of inflammatory responses.

Evaluation of biocompatibility

To evaluate the biocompatibility of MTBN, its cytotoxicity toward RAW264.7 macrophages and L929 fibroblasts was first assessed using a CCK-8 assay. In RAW264.7 cells, BF exhibited dose-dependent toxicity, reducing cell viability to 61.8% at 4 μ g/mL and 42.5% at 16 μ g/mL, with a toxic response already evident at 2 μ g/mL. In contrast, the clinically used antibiotic Tob showed no detectable cytotoxicity across the tested concentrations. Notably, MTBN maintained high viability even at 16 μ g/mL (89.7%), indicating that MTBN effectively attenuated BF-associated cytotoxicity. A similar trend was observed in L929 cells, where MTBN preserved 79.6% viability at 16 μ g/mL (Fig. S19A). Blood compatibility was then examined by hemolysis testing. As shown in Fig. S19B, MTBN induced minimal hemolysis (0.56%) even at 64 μ g/mL, suggesting negligible damage to red blood cells. Finally, *in vivo* safety was evaluated by H&E staining of major organs. Compared with healthy controls, the PBS-treated group displayed inflammatory cell infiltration and hemorrhagic features in the liver, spleen, and kidney, along with enlarged splenic lymphoid regions. In contrast, no evident histopathological abnormalities were observed in MTBN-treated mice (Fig. S19C). Collectively, these results demonstrate that MTBN exhibits favorable biocompatibility and a good safety profile both *in vitro* and *in vivo*, supporting its potential as an effective therapeutic nanodrug platform for intracellular bacterial infections.

Conclusion

In this study, a mannose-functionalized, carrier-free nanodrug (MTBN) is developed to address intracellular bacterial infections by integrating active targeting, microenvironment-triggered disassembly, and synergistic antibacterial action. MTBN is preferentially internalized by infected macrophages via mannose-receptor-mediated endocytosis. In acidic lysosomes, the acid-labile mannose linkage dissociates to expose cationic amino groups, which promotes lysosomal escape. Once in the mildly acidic intracellular infection niche, MTBN disassembles into smaller nanofragments that facilitate bacterial internalization and increase intrabacterial drug availability. Meanwhile, Schiff-base cleavage enables Tob and BF to act in concert. Through this cascade process, bacterial membrane integrity is severely compromised and durable bactericidal activity is achieved. Consistent with these mechanistic features, MTBN exhibits markedly improved intracellular antibacterial efficacy *in vitro* and *in vivo* compared with free drugs, their physical mixtures, and non-targeted carrier-free nanodrugs, while exhibiting negligible systemic toxicity. This cascade-targeting strategy establishes a clinically translatable paradigm for refractory intracellular infections and advances the broader utility of carrier-free nanomedicines.

Materials and methods

Materials

4-Formylbenzoic acid ($\geq 98\%$) was purchased from Bide Pharmatech Co., Ltd. (China). (-)-Borneol ($\geq 97\%$) was obtained from Aladdin Biochemical Technology Co., Ltd. (China). N,N'-Dicyclohexylcarbodiimide (DCC, $\geq 98\%$), 4-dimethylaminopyridine (DMAP, $\geq$

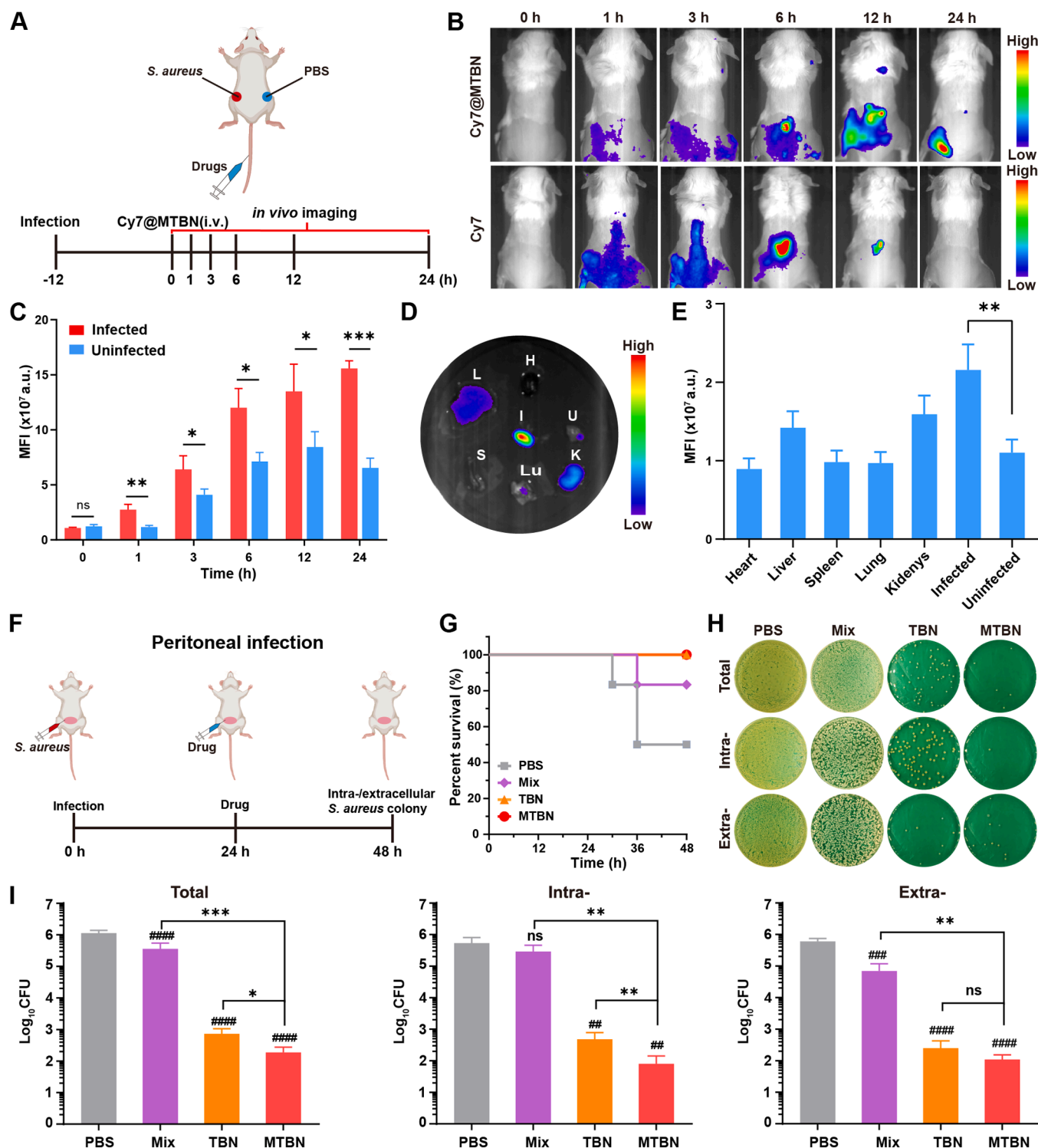


Fig. 7. *In vivo* targeting and antibacterial performance of MTBN. (A) Schematic illustration of the mouse muscle infection model. (B) Representative fluorescence images of infected mice. (C) Quantitative fluorescence analysis of infected and uninfected sites ($n = 3$). (D) *Ex vivo* fluorescence images of major organs and tissues collected 24 h post-injection of *Cy7@MTBN*, including heart, liver, spleen, lung, kidney, infected site, and uninfected site, abbreviated as H, L, S, Lu, K, I, and U, respectively. (E) Quantitative MFI analysis of *ex vivo* organs and tissues ($n = 3$). (F) Schematic illustration of the mouse peritonitis infection model. (G) Survival curves of mice during the treatment of peritonitis infection ($n = 6$). (H) Representative images of total, intracellular, and extracellular bacterial colonies on agar plates after 24 h of treatment with different formulations. (I) Quantitative CFU analysis of total, intracellular, and extracellular bacteria at 48 h using surviving animals: PBS group, $n = 3$; Mix group, $n = 5$; TBN and MTBN groups, $n = 6$. # indicates statistical significance between the experimental group and the control group.

99%), and D-(+)-mannose ($\geq 98\%$) were purchased from Tokyo Chemical Industry Co., Ltd. (TCI, Japan). Tobramycin ($\geq 94\%$), coumarin-6 ($\geq 98\%$), ethyl acetate, tetrahydrofuran (THF), petroleum

ether, sodium hydroxide, and dichloromethane (DCM) were obtained from J&K Scientific Ltd. (China). Nile Red ($\geq 95\%$) was purchased from Shanghai Macklin Biochemical Co., Ltd. (China). Tryptic Soy Broth

(TSB) and Tryptic Soy Agar (TSA) were obtained from China National Pharmaceutical Group Corporation (China). The Live/Dead bacterial viability kit, CCK-8, LysoTracker Green, 4',6-diamidino-2-phenylindole (DAPI), Hanks' balanced salt solution (HBSS), bovine serum albumin (BSA), lysozyme, and Triton X-100 were purchased from Beijing Solarbio Science & Technology Co., Ltd. (China). Cell culture media were obtained from Gibco BRL (USA) and Corning Inc. (USA).

Characterization

^1H NMR spectra were recorded at room temperature using DMSO- d_6 as the solvent on a nuclear magnetic resonance spectrometer (400 MHz, Bruker, Germany). Fourier-transform infrared (FTIR) spectra were obtained to identify chemical functional groups using an FTIR spectrometer (Nicolet 8700, Thermo Fisher Scientific, USA.). X-ray photoelectron spectroscopy (XPS) measurements were performed using an XPS spectrometer (ESCALAB 250, Thermo Fisher Scientific, USA.). The hydrodynamic diameter and zeta potential of the nanodrugs were determined by dynamic light scattering using a particle size and zeta potential analyzer (Zetasizer Nano ZS90, Malvern Instruments, U.K.). The morphology of nanodrugs and bacteria was examined by transmission electron microscopy (HT-7700, Hitachi, Japan) and scanning electron microscopy (JSM-7800F, JEOL, Japan). UV-vis spectroscopy was performed using a UV-vis spectrophotometer (UV-2450, Shimadzu, Japan) to quantify drug loading and monitor drug-release profiles. Confocal laser scanning microscopy (CLSM) was conducted using a confocal microscope (TCS SP8, Leica, Germany) to evaluate cellular uptake, bacterial live/dead staining, lysosomal escape, and bacterial targeting. Flow cytometry was performed using a flow cytometer (CytoFLEX, Beckman Coulter, USA.) to analyze cellular uptake and macrophage polarization. In vivo fluorescence imaging of mice was conducted using an IVIS imaging system (IVIS Spectrum, PerkinElmer, USA.).

MTBN synthesis

Synthesis of BF

BF was synthesized via a previously reported esterification of 4-formylbenzoic acid with borneol. [51] 4-Formylbenzoic acid (1.45 g) was dissolved in anhydrous THF (30 mL) at 25 °C, followed by the addition of DMAP (0.15 g) and DCC (2.6 g). After complete dissolution, borneol (1 g) was added, and the reaction mixture was stirred at room temperature for 24 h. The precipitate was removed by vacuum filtration, and the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography using petroleum ether/ethyl acetate (10:1, v/v) to afford BF after vacuum drying.

Synthesis of MTBN

TBN was prepared via oil-water interfacial Schiff-base reaction. BF (30 mg) was dissolved in DCM (1 mL), followed by the addition of deionized water (3.4 mL) under stirring (1600 rpm) for 6 h to form a stable emulsion. Tob solution (1.6 mL, 3% w/v) was then added dropwise, and the mixture was further stirred at 1600 rpm for 4 h. After solvent evaporation, the dispersion was dialyzed against deionized water (MWCO 1000 Da) for 48 h to obtain TBN. MTBN was prepared by reacting TBN with mannose in NaOH solution (6 mL, 1.5% w/v, pH 12) at 60 °C and 800 rpm for 2 h, followed by centrifugal ultrafiltration (MWCO 3500 Da) to remove excess mannose [66]. Fluorescently labeled formulations, including NR@TBN/NR@MTBN, C6@TBN/C6@MTBN, Cy7@TBN/Cy7@MTBN, I@TBN and AIE@MTBN was prepared by dissolving the corresponding dye in the BF/DCM phase before emulsification, followed by the same preparation and purification procedures.

Drug loading determination

Tob content was quantified using an o-phthalaldehyde (OPA)

colorimetric assay. The OPA working reagent was prepared by dissolving OPA (100 mg) in borax buffer (pH 10.5), followed by the addition of β -Mercaptoethanol (1 mL), adjustment to pH 10.5, and dilution to 100 mL. For analysis, sample solution (1 mL) was mixed with OPA working reagent (1 mL) and isopropanol (3 mL), incubated at 60 °C for 15 min, cooled to room temperature, and measured at 333 nm by UV-vis spectroscopy [50]. Residual Tob in the TBN dialysate was quantified to calculate Tob loading. Mannose content was determined using the sulfuric acid-phenol colorimetric assay [51]. Mannose solution (30 μL) was mixed with 5% phenol (30 μL) and concentrated sulfuric acid (100 μL), incubated at 90 °C for 1 h, cooled to room temperature, and measured at 490 nm by UV-vis spectroscopy. A mannose standard curve was used to quantify residual mannose in the MTBN ultrafiltrate for calculation of mannose loading. Drug loading content (wt%) was calculated as follows:

$$\text{DLE(wt\%)}\text{ of Tob} = \frac{m(\text{Tob})}{m(\text{MTBN})} \times 100\% \quad (1)$$

$$\text{DLE(wt\%)}\text{ of BF} = \frac{m(\text{BF})}{m(\text{MTBN})} \times 100\% \quad (2)$$

$$\text{DLE(wt\%)}\text{ of Mannose} = \frac{m(\text{Man})}{m(\text{MTBN})} \times 100\% \quad (3)$$

Release Profile of Tobramycin

The release profiles of TBN and MTBN were evaluated by dialysis. Briefly, 2 mL of each formulation was sealed in a dialysis bag (MWCO 3500 Da) and immersed in 15 mL of PBS (pH 7.4 or 5.5) at 37 °C with shaking (100 rpm). At predetermined intervals, 1 mL of release medium was withdrawn and replaced with an equal volume of fresh buffer. Tob content in the collected samples was determined by the OPA assay, and the cumulative release was calculated accordingly. All release assay was conducted with three independent replicates.

Extracellular antibacterial activity assay

MICs were determined by broth microdilution. Twofold serial dilutions of each formulation were prepared in TSB in 96-well plates (100 μL /well) and incubated with *S. aureus* (1×10^6 CFU/mL) at 37 °C for 24 h. Bacterial growth was measured at 600 nm, and the MIC was defined as the lowest concentration showing no visible growth relative to the untreated control. MBCs were determined by plate counting after incubation of *S. aureus* (1×10^6 CFU/mL) with the indicated formulations at 37 °C for 24 h, followed by plating of aliquots (100 μL) on TSA; the MBC was defined as the lowest concentration producing no detectable colonies. For extracellular CFU counting, *S. aureus* (1×10^6 CFU/mL) was treated with MTBN, TBN, Mix, BF, or Tob at 37 °C for 24 h and plated on TSA. Concentrations were normalized to the component contents in MTBN (1 μg /mL), TBN (0.85 μg /mL), and Mix at the same molar ratio as MTBN (BF 0.47 μg /mL, Tob 0.38 μg /mL, and mannose 0.15 μg /mL), while BF and Tob alone were tested at 0.47 and 0.38 μg /mL, respectively.

Growth inhibition curve

To evaluate the growth kinetics of *S. aureus* under different treatments, bacterial suspensions (1×10^8 CFU/mL) were incubated with PBS, BF, Tob, Mix, TBN, or MTBN at 37 °C. Each formulation was tested at concentrations corresponding to $0.5 \times$, $1 \times$, and $2 \times$ MIC. At designated time points, 100 μL of the bacterial culture was withdrawn, and OD (600 nm) was recorded to generate growth curves.

Live/Dead bacterial staining

S. aureus (1×10^8 CFU/mL) was incubated with each formulation at 37 °C for 24 h. Concentrations were normalized to the component doses in MTBN (2.0 µg/mL): MTBN (2.0 µg/mL), TBN (1.7 µg/mL), Mix (BF 0.94 µg/mL, Tob 0.76 µg/mL, and mannose 0.30 µg/mL), with BF and Tob alone tested at 0.94 and 0.76 µg/mL, respectively. After treatment, bacteria were collected by centrifugation (9000 g, 3 min), washed three times with PBS, and stained using Live/Dead bacterial viability kit (AO/PI). Fluorescence images were obtained by CLSM, and fluorescence intensities were analyzed using ImageJ.

Bacterial SEM analysis

S. aureus (1×10^8 CFU/mL) was incubated with PBS, BF, Tob, Mix, TBN, or MTBN at 37 °C for 6 h, with drug concentrations normalized to the component-equivalent dose in MTBN (2.0 µg/mL). Bacteria were then collected by centrifugation (7500 rpm, 3 min), washed three times with PBS, and fixed with 2.5% glutaraldehyde at 4 °C for 2 h. After graded ethanol dehydration (30–100%), samples were deposited onto silicon wafers, air-dried, and imaged by SEM.

Bacterial-nanodrugs colocalization

GFP-*S. aureus* in the logarithmic phase was diluted in TSB to 1×10^6 CFU/mL and co-incubated with NR@MTBN (Nile Red, 10 µg/mL) at 37 °C for 2 h. After incubation, bacteria were washed three times with PBS and imaged by CLSM.

Zeta potential measurement of bacteria

S. aureus was dispersed in PBS at 1×10^9 CFU/mL and incubated with MTBN at the indicated concentrations (2 or 4 µg/mL) at 37 °C for 2 h with shaking (100 rpm). The bacteria were then collected by centrifugation (7500 rpm, 3 min), washed twice with PBS, resuspended in deionized water, and analyzed for surface zeta potential.

Synchrotron X-ray imaging of bacteria

S. aureus (1×10^9 CFU/mL) was incubated with I@TBN (2 µg/mL) for 1 h, washed three times with PBS, and fixed with glutaraldehyde, untreated bacteria were used as the control. Aliquots (10 µL) were deposited onto TEM nickel grids, allowed to settle for 5 min, rinsed twice with deionized water, and plunge-frozen for synchrotron X-ray imaging. Tomographic data were collected at 520 eV with a 1° rotation step over a 9.5 µm field of view. The datasets were reconstructed using the simultaneous iterative reconstruction technique (SIRT), yielding a pixel size of 19.3 nm.

Evaluation of synergistic antibacterial activity

Checkerboard assay

BF and Tob were twofold serially diluted along the orthogonal axes of a 96-well plate to generate a matrix of drug combinations. *S. aureus* suspension (5×10^5 CFU/mL) was added to each well, and the plate was incubated at 37 °C for 24 h. Bacterial growth was evaluated by measuring OD (600 nm). The fractional inhibitory concentration index (FICI) was calculated according to Eq. (4).

$$FICI = \frac{MIC_A \text{ in combination}}{MIC_A} + \frac{MIC_B \text{ in combination}}{MIC_B} \quad (4)$$

Synergy was interpreted as follows: $FICI \leq 0.5$, synergistic; $0.5 < FICI \leq 0.75$, partially synergistic; $0.75 \leq FICI < 2$, no interaction; and $FICI \geq 2$, antagonistic [67].

Mannose-enhanced tobramycin antibacterial activity

2 µg/mL Tob was dispensed into a 96-well plate and mixed with twofold serial dilutions of mannose. *S. aureus* was prepared in M9 medium (1×10^6 CFU/mL) and added to each well, followed by incubation at 37 °C for 24 h [55]. After incubation, bacterial suspensions were serially diluted in PBS and spread onto TSA plates. Colonies were counted after incubation, and bacterial survival was calculated for each group.

Bacterial membrane depolarization assay

5 mM DiOC₂(3) stock solution was prepared in anhydrous DMSO and diluted with PBS to obtain a 30 µM working solution. *S. aureus* (1×10^9 CFU/mL) was incubated with the indicated treatments (MTBN 2 µg/mL, TBN 1.72 µg/mL, Mix 2 µg/mL, Tob 0.76 µg/mL, and BF 0.94 µg/mL) at 37 °C for 24 h. After treatment, 100 µL of each bacterial suspension was transferred to a black 96-well plate and mixed with 10 µL of the DiOC₂(3) working solution. The plate was incubated at room temperature for 15 min in the dark, and fluorescence was recorded using a microplate reader (Ex/Em = 482/497 nm).

Bacterial membrane permeability assay

ONPG solution (0.015 g/mL) was prepared in physiological saline. *S. aureus* suspensions (1×10^9 CFU/mL) were treated with the indicated formulations (MTBN 2 µg/mL, TBN 1.72 µg/mL, Mix 2 µg/mL, Tob 0.76 µg/mL, and BF 0.94 µg/mL) at 37 °C for 1 h with shaking at 180 rpm. After treatment, bacteria were collected by centrifugation (7500 rpm, 3 min) and washed twice with saline. The pellets were then resuspended in ONPG solution and incubated for 3 h in a thermostatic shaker. Following incubation, samples were centrifuged again (7500 rpm, 3 min), and the absorbance of the supernatant was measured at 420 nm using a microplate reader.

Protein leakage assay

S. aureus (1×10^9 CFU/mL) was incubated with the indicated formulations at concentrations equivalent to that of MTBN (4 µg/mL) at 37 °C for 6 h. After centrifugation (7500 rpm, 3 min), 20 µL of sample was mixed with 200 µL of BCA working reagent, followed by incubation at 37 °C for 1 h. Absorbance at 562 nm was measured, and protein concentration was determined using a BSA standard curve ($R^2 \geq 0.995$). Relative protein leakage was calculated according to Eq. (5).

$$\text{Relative leakage rate(\%)} = \frac{\text{CONC. Sample} - \text{CONC. Blank}}{\text{CONC. RIPA} - \text{CONC. Blank}} \quad (5)$$

Cellular internalization and intracellular trafficking of nanodrugs

Cellular uptake

RAW264.7 macrophages were seeded in confocal dishes (2×10^5 cells/mL) and incubated with coumarin-6-labeled nanodrugs (C6@MTBN or C6@TBN) or free coumarin-6 (5 µg/mL) for the indicated times. Cells were then washed with PBS, fixed with 2.5% glutaraldehyde and 2% paraformaldehyde, stained with DAPI, and imaged by CLSM.

Mannose competitive uptake

RAW264.7 macrophages were preincubated with mannose (1 mg/mL) for 1 h before exposure to C6@TBN or C6@MTBN (C6: 5 µg/mL) for 3 h. After washing and fixation, samples were stained with DAPI and analyzed by CLSM and flow cytometry (Ex/Em = 406/505 nm).

Intracellular colocalization of nanodrugs with lysosomes

RAW264.7 macrophages were cultured for 24 h and then incubated with AIE@MTBN for 1, 3, 6, or 12 h. Lysosomes were stained with

LysoTracker Green for 1 h, and cells were washed and imaged by CLSM to assess intracellular distribution and lysosomal colocalization.

Intracellular bacteria and nanodrugs colocalization

The intracellular infection model was established using GFP-*S. aureus*. RAW264.7 macrophages were seeded in 24-well plates at 1×10^5 cells/well and cultured for 18–24 h. GFP-*S. aureus* suspensions (1×10^6 CFU/mL in antibiotic-free RPMI 1640 medium) were then added at an MOI of 10 and incubated for 1 h. After washing with PBS, the infected cells were treated with NR@MTBN for 24 h, fixed with 2.5% glutaraldehyde for 1 h, stained with DAPI, and imaged by CLSM in PBS.

In vitro intracellular antibacterial evaluation

S. aureus-infected RAW264.7 macrophages (MOI: 10) were treated with PBS, BF, Tob, Mix, TBN, or MTBN at doses equivalent to MTBN (16 μ g/mL) for 12, 24, or 48 h. Cells were then lysed with PBS containing 0.1% Triton X-100, and the released intracellular bacteria were serially diluted, plated on TSA agar, and incubated at 37 °C for 24 h for colony counting.

Macrophage polarization analysis

Macrophage polarization was evaluated by flow cytometry. *S. aureus*-infected RAW264.7 macrophages were treated with PBS, Tob, Mix, TBN, or MTBN for 24 h, harvested, and stained with PE-conjugated anti-mouse CD80 and FITC-conjugated anti-mouse CD206 antibodies. Fluorescence signals were then analyzed by flow cytometry.

In vivo targeting and distribution

8 weeks old female Balb/c mice were used to establish the murine thigh muscle infection model. Briefly, *S. aureus* suspension (10^8 CFU in 100 μ L of PBS) was intramuscularly injected into the left thigh, while the contralateral thigh was injected with 100 μ L of PBS as an uninfected control. After 12 h, mice were intravenously injected with Cy7@MTBN or free Cy7. Whole-body fluorescence imaging was performed at pre-determined time points using an IVIS system (Ex/Em = 743/767 nm). At 24 h post-injection, mice were sacrificed, and major organs were collected for ex vivo fluorescence imaging.

In vivo antibacterial activity

8 weeks old female Balb/c mice were intraperitoneally injected with *S. aureus* suspension (10^8 CFU in 100 μ L of PBS) to establish the peritonitis infection model. After 24 h, the infected mice were treated intraperitoneally with PBS, Mix, TBN, or MTBN, with all drug doses normalized to the Tob content in MTBN (30 mg/kg). At 24 h after treatment, mice were intraperitoneally injected with 2 mL of HBSS and then euthanized. The peritoneal lavage fluid was collected for quantification of total, extracellular, and intracellular bacteria. Briefly, one-third of the peritoneal fluid was used directly to determine total CFUs. Another third was centrifuged, and the supernatant was collected to quantify extracellular CFUs. The remaining third was incubated with lysozyme (15 μ g/mL) to eliminate extracellular bacteria, followed by lysis with HBSS containing 0.1% Triton X-100 to release intracellular bacteria for CFU counting. Blood samples were collected for serum analysis of pro-inflammatory markers, including LDH and HS-CRP. Major organs, including the heart, liver, spleen, lung, and kidney, were harvested for histopathological evaluation by H&E staining.

Biosafety evaluation

Cytotoxicity assessment

The cytotoxicity of BF, Tob, and MTBN toward L929 and RAW264.7

cells was evaluated using a CCK-8 assay. Cells were seeded in 96-well plates at 8×10^3 cells per well and cultured for 18–24 h. The medium was then replaced with fresh medium containing twofold serial dilutions of each formulation. After 24 h of incubation, cells were washed with PBS, treated with CCK-8 working solution for 1 h, and measured at 540 nm using a microplate reader. Cell viability was calculated according to Eq. (6).

$$\text{Cell viability(\%)} = \frac{(\text{Abs}_{\text{samples}} - \text{Abs}_{\text{blank}})}{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{blank}})} \times 100\% \quad (6)$$

Hemolysis assay

Anticoagulated mouse blood was diluted with PBS and centrifuged (500 $\times$ g, 10 min). The erythrocytes were washed five times with PBS and resuspended to obtain a red blood cell suspension. MTBN dispersions at different concentrations were mixed with the red blood cell suspension and incubated at 37 °C with gentle shaking (30 rpm) for 3 h. After centrifugation (10,000 $\times$ g, 5 min), the absorbance of the supernatants was measured at 540 nm using a microplate reader. Hemolysis was calculated according to Eq. (7).

$$\text{Hemolysis(\%)} = \frac{(\text{Abs}_{\text{samples}} - \text{Abs}_{\text{negative}})}{(\text{Abs}_{\text{positive}} - \text{Abs}_{\text{negative}})} \times 100\% \quad (7)$$

where $\text{Abs}_{\text{samples}}$, $\text{Abs}_{\text{negative}}$ and $\text{Abs}_{\text{positive}}$ represent the absorbance of the sample, negative control, and positive control, respectively.

Statistical analysis

Colocalization analysis and gap area calculation were performed by using the ImageJ software. All data were presented as mean $\pm$ standard deviation (SD) of independent biological replicates. Statistical analyses were performed using GraphPad Prism, the statistical significance of differences was determined by Student's *t*-test or one-way ANOVA. */# $p < 0.05$, **/## $p < 0.01$, ***/### $p < 0.001$, ****/#### $p < 0.0001$, ns $p > 0.05$.

CRediT authorship contribution statement

Runyu Han: Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Yanan Fu:** Methodology, Investigation, Conceptualization. **Yuecong Guo:** Visualization, Investigation. **Yizhen You:** Investigation. **Jiali Yang:** Investigation. **Yaling Wang:** Resources, Investigation. **Wensheng Xie:** Supervision, Resources, Funding acquisition. **Guofeng Li:** Writing – review & editing, Supervision, Resources, Funding acquisition. **Xing Wang:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Ethics approval and consent to participate

All animals were treated and cared for in accordance with the National Research Council's Guide for the care and use of laboratory animals and under supervision and assessment by the Experimental Animal Ethics Committee of Beijing University of Chemical Technology (Approval No: BUCT 21–2512–082).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This research was supported by the National Natural Science

Foundation of China (52273118, 22275013), the Prevention and Control of Emerging and Major Infectious Diseases-National Science and Technology Major Project (2025ZD01902201), the Fundamental Research Funds for the Central Universities (buctrc202419, PY2508), the Innovation & Transfer Fund of Peking University Third Hospital (BYSYZHKC123, BYSYZHKC107), and the Interdisciplinary Research Center of Beijing University of Chemical Technology (No. XK2025-05).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.nantod.2026.103120](https://doi.org/10.1016/j.nantod.2026.103120).

Data availability

Data will be made available on request.

References

- [1] E.A. Masters, B.F. Ricciardi, K.L.D.M. Bentley, T.F. Moriarty, E.M. Schwarz, G. Muthukrishnan, Skeletal infections: microbial pathogenesis, immunity and clinical management, *Nat. Rev. Microbiol.* 20 (2022) 385–400, <https://doi.org/10.1038/s41579-022-00686-0>.
- [2] P. Santucci, D.J. Greenwood, A. Fearn, K. Chen, H. Jiang, M.G. Gutierrez, Intracellular localisation of mycobacterium tuberculosis affects efficacy of the antibiotic pyrazinamide, *Nat. Commun.* 12 (2021) 3816, <https://doi.org/10.1038/s41467-021-24127-3>.
- [3] G.E. Thwaites, V. Gant, Are bloodstream leukocytes trojan horses for the metastasis of *Staphylococcus aureus*? *Nat. Rev. Microbiol.* 9 (2011) 215–222, <https://doi.org/10.1038/nrmicro2508>.
- [4] K. Ray, B. Marteyn, P.J. Sansonetti, C.M. Tang, Life on the inside: the intracellular lifestyle of cytosolic bacteria, *Nat. Rev. Microbiol.* 7 (2009) 333–340, <https://doi.org/10.1038/nrmicro2112>.
- [5] S.H.E. Kaufmann, Intracellular pathogens: living in an extreme environment, *Immunol. Rev.* 240 (2011) 5–10, <https://doi.org/10.1111/j.1600-065X.2010.01001.x>.
- [6] D.M. Sivaloganathan, M.P. Brynildsen, Phagosome–bacteria interactions from the bottom up, *Annu. Rev. Chem. Biomol. Eng.* 12 (2021) 309–331, <https://doi.org/10.1146/annurev-chembioeng-090920-015024>.
- [7] D.G. Russell, L. Huang, B.C. VanderVen, Immunometabolism at the interface between macrophages and pathogens, *Nat. Rev. Immunol.* 19 (2019) 291–304, <https://doi.org/10.1038/s41577-019-0124-9>.
- [8] K. Stelzner, A.-C. Winkler, C. Liang, A. Boyny, C.P. Ade, T. Dandekar, M. J. Fraunholz, T. Rudel, Intracellular *Staphylococcus aureus* perturbs the host cell Ca²⁺ homeostasis to promote cell death, *10.1128/mbio.02250-20*, *mBio* 11 (2020), <https://doi.org/10.1128/mbio.02250-20>.
- [9] J. Horn, K. Stelzner, T. Rudel, M. Fraunholz, Inside job: *Staphylococcus aureus* host-pathogen interactions, *Int. J. Med. Microbiol.* 308 (2018) 607–624, <https://doi.org/10.1016/j.ijmm.2017.11.009>.
- [10] J. Jubrail, P. Morris, M.A. Bewley, S. Stoneham, S.A. Johnston, S.J. Foster, A. A. Peden, R.C. Read, H.M. Marriott, D.H. Dockrell, Inability to sustain intraphagolysosomal killing of *Staphylococcus aureus* predisposes to bacterial persistence in macrophages, *Cell. Microbiol.* 18 (2016) 80–96, <https://doi.org/10.1111/cmi.12485>.
- [11] K. S. Ikuta, L.R. Swetschinski, G.R. Aguilar, et al., Global mortality associated with 33 bacterial pathogens in 2019: a systematic analysis for the global burden of disease study 2019, *Lancet* 400 (2022) 2221–2248, [https://doi.org/10.1016/S0140-6736\(22\)02185-7](https://doi.org/10.1016/S0140-6736(22)02185-7).
- [12] T. Vos, S.S. Lim, C. Abbafati, et al., Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: a systematic analysis for the global burden of disease study 2019, *Lancet* 396 (2020) 1204–1222, [https://doi.org/10.1016/S0140-6736\(20\)30925-9](https://doi.org/10.1016/S0140-6736(20)30925-9).
- [13] S.M. Lehar, T. Pillow, M. Xu, et al., Novel antibody–antibiotic conjugate eliminates intracellular *S. aureus*, *Nature* 527 (2015) 323–328, <https://doi.org/10.1038/nature16057>.
- [14] F. Peyrussion, H. Varet, T.K. Nguyen, R. Legendre, O. Sismeiro, J.-Y. Coppée, C. Wolz, T. Tenson, F. Van Bambeke, Intracellular *Staphylococcus aureus* persists upon antibiotic exposure, *Nat. Commun.* 11 (2020) 2200, <https://doi.org/10.1038/s41467-020-15966-7>.
- [15] M. Barcia-Macay, C. Seral, M.-P. Mingot-Leclercq, P.M. Tulkens, F. Van Bambeke, Pharmacodynamic evaluation of the intracellular activities of antibiotics against *Staphylococcus aureus* in a model of THP-1 macrophages, *Antimicrob. Agents Chemother.* 50 (2006) 841–851, <https://doi.org/10.1128/aac.50.3.841-851.2006>.
- [16] S. Subramaniam, P. Joyce, N. Thomas, C.A. Prestidge, Bioinspired drug delivery strategies for repurposing conventional antibiotics against intracellular infections, *Adv. Drug. Deliv. Rev.* 177 (2021) 113948, <https://doi.org/10.1016/j.addr.2021.113948>.
- [17] J.-X. Yuan, Y.-J. Gao, R. Hua, T.-C. Wei, G.-Y. Xu, J. Zhou, K. Zhang, Y.-T. Li, J.-Q. Feng, W.-J. Lai, L. Wang, X. Chen, Antimicrobial effects of fibrous unit in modular self-assembling peptides, *Nano Today* 65 (2025) 102860, <https://doi.org/10.1016/j.nantod.2025.102860>.
- [18] J. He, M. Hong, W. Xie, Z. Chen, D. Chen, S. Xie, Progress and prospects of nanomaterials against resistant bacteria, *J. Control. Release* 351 (2022) 301–323, <https://doi.org/10.1016/j.jconrel.2022.09.030>.
- [19] R.S. Santos, C. Figueiredo, N.F. Azevedo, K. Braeckmans, S.C. De Smedt, Nanomaterials and molecular transporters to overcome the bacterial envelope barrier: towards advanced delivery of antibiotics, *Adv. Drug. Deliv. Rev.* 136–137 (2018) 28–48, <https://doi.org/10.1016/j.addr.2017.12.010>.
- [20] N. Abed, P. Couvreur, Nanocarriers for antibiotics: a promising solution to treat intracellular bacterial infections, *Int. J. Antimicrob. Agents* 43 (2014) 485–496, <https://doi.org/10.1016/j.ijantimicag.2014.02.009>.
- [21] M. Wang, R. Li, S. Sheng, H. Yang, X. Tang, J. Wang, F. Wang, Q. Zhang, L. Bai, X. Chen, J. Gao, X. Ren, H. Liu, J. Su, MOF nanzyme mediated bacterial metabolic regulation to intervene MRSA antibiotic tolerance for enhanced antimicrobial efficacy, *Nano Today* 63 (2025) 102753, <https://doi.org/10.1016/j.nantod.2025.102753>.
- [22] S.M. Hosseini, M. Taheri, F. Nouri, A. Farmani, N.M. Moez, M.R. Arabestani, Nano drug delivery in intracellular bacterial infection treatments, *Biomed. Pharmacother.* 146 (2022) 112609, <https://doi.org/10.1016/j.biopha.2021.112609>.
- [23] W. Feng, M. Chittò, T.F. Moriarty, G. Li, X. Wang, Targeted drug delivery systems for eliminating intracellular bacteria, *Macromol. Biosci.* 23 (2023) 2200311, <https://doi.org/10.1002/mabi.202200311>.
- [24] W. Feng, G. Li, X. Kang, R. Wang, F. Liu, D. Zhao, H. Li, F. Bu, Y. Yu, T.F. Moriarty, Q. Ren, X. Wang, Cascade-targeting poly(amino acid) nanoparticles eliminate intracellular bacteria via on-site antibiotic delivery, *Adv. Mater.* 34 (2022) 2109789, <https://doi.org/10.1002/adma.202109789>.
- [25] J.-Y. Gong, Y. Li, P. Wang, X. Peng, R. Xie, W. Wang, Z. Liu, D.-W. Pan, X.-J. Ju, L.-Y. Chu, K⁺-responsive nanoparticles with charge reversal and gating synergistic effects for targeted intracellular bacteria eradication, *ACS Nano* 19 (2025) 2345–2361, <https://doi.org/10.1021/acsnano.4c12542>.
- [26] Y. Cai, M. Qiao, X. Xie, Y. Zhou, R. Yu, F. Song, H. Wang, H. Zhang, X. Sun, Intracellular MRSA-targeted lipid-polymer hybrid nanoparticles for macrophage reprogramming and intracellular MRSA eradication, *Chem. Eng. J.* 505 (2025) 159665, <https://doi.org/10.1016/j.cej.2025.159665>.
- [27] W. Wang, W. Mo, X. Xiao, M. Cai, S. Feng, Y. Wang, D. Zhou, Antibiotic-loaded lactoferrin nanoparticles as a platform for enhanced infection therapy through targeted elimination of intracellular bacteria, *Asian J. Pharm. Sci.* 19 (2024) 100926, <https://doi.org/10.1016/j.ajps.2024.100926>.
- [28] S. Xie, Y. Li, W. Cao, J. Peng, K. Huang, J. Meng, X. Li, Dual-responsive nanogels with cascaded gentamicin release and lysosomal escape to combat intracellular small colony variants for peritonitis and sepsis therapies, *Adv. Healthc. Mater.* 13 (2024) 2303671, <https://doi.org/10.1002/adhm.202303671>.
- [29] Y.-C. Yeh, T.-H. Huang, S.-C. Yang, C.-C. Chen, J.-Y. Fang, Nano-based drug delivery or targeting to eradicate bacteria for infection mitigation: A review of recent advances, *Front. Chem.* 8 (2020) 286, <https://doi.org/10.3389/fchem.2020.00286>.
- [30] S. Hossen, M.K. Hossain, M.K. Basher, M.N.H. Mia, M.T. Rahman, M.J. Uddin, Smart nanocarrier-based drug delivery systems for cancer therapy and toxicity studies: A review, *J. Adv. Res.* 15 (2019) 1–18, <https://doi.org/10.1016/j.jare.2018.06.005>.
- [31] S. Đorđević, M.M. Gonzalez, I. Conejos-Sánchez, B. Carreira, S. Pozzi, R.C. Acúrcio, R. Satchi-Fainaro, H.F. Florindo, M.J. Vicent, Current hurdles to the translation of nanomedicines from bench to the clinic, *Drug. Deliv. Transl. Res.* 12 (2022) 500–525, <https://doi.org/10.1007/s13346-021-01024-2>.
- [32] R.V. Kumarasamy, P.M. Natarajan, V.R. Umapathy, J.R. Roy, M. Mironescu, C. P. Palanisamy, Clinical applications and therapeutic potentials of advanced nanoparticles: a comprehensive review on completed human clinical trials, *Front. Nanotechnol.* 6 (2024) 1–25, <https://doi.org/10.3389/fnano.2024.1479993>.
- [33] S. Milewska, K. Niemirówicz-Laskowska, G. Siemiaszko, P. Nowicki, A. Z. Wilczewska, H. Car, Current trends and challenges in pharmacoeconomic aspects of nanocarriers as drug delivery systems for cancer treatment, *Int. J. Nanomed.* 16 (2021) 6593–6644, <https://doi.org/10.2147/IJN.S323831>.
- [34] H. Mei, S. Cai, D. Huang, H. Gao, J. Cao, B. He, Carrier-free nanodrugs with efficient drug delivery and release for cancer therapy: from intrinsic physicochemical properties to external modification, *Bioact. Mater.* 8 (2022) 220–240, <https://doi.org/10.1016/j.bioactmat.2021.06.035>.
- [35] F. Fang, X. Chen, Carrier-free nanodrugs: from bench to bedside, *ACS Nano* 18 (2024) 23827–23841, <https://doi.org/10.1021/acsnano.4c09027>.
- [36] X. Dong, H. Liu, H. Liu, X. Zhang, X. Deng, Carrier-free nanomedicines: mechanisms of formation and biomedical applications, *Giant* 18 (2024) 100256, <https://doi.org/10.1016/j.giant.2024.100256>.
- [37] S.-Y. Qin, A.-Q. Zhang, S.-X. Cheng, L. Rong, X.-Z. Zhang, Drug self-delivery systems for cancer therapy, *Biomaterials* 112 (2017) 234–247, <https://doi.org/10.1016/j.biomaterials.2016.10.016>.
- [38] X. Zhang, S. Hu, L. Huang, X. Chen, X. Wang, Y. Fu, H. Sun, G. Li, X. Wang, Advance progress in assembly mechanisms of carrier-free nanodrugs for cancer treatment, *Molecules* 28 (2023) 7065, <https://doi.org/10.3390/molecules28207065>.
- [39] Y. Fu, J. Yang, L. Huang, F. Liu, F. Bu, X. Kang, G. Li, Q. Ren, T.F. Moriarty, X. Wang, Self-carrier nanodrugs with dual antibacterial agents to overcome tobramycin resistance in multidrug-resistant *Staphylococcus aureus*, *Bioact. Mater.* 56 (2026) 386–401, <https://doi.org/10.1016/j.bioactmat.2025.10.022>.
- [40] J. Xiang, M. Suo, J. Lan, S. Ning, H. Liu, W. Hong, J. Liang, J. Zheng, D. Zhu, J. Mo, Novel carrier-free nanomedicine for regulating macrophage phenotype to amplify

- anti-tumor photoimmunotherapy, *Small* 21 (2025), <https://doi.org/10.1002/smll.202505304>.
- [41] L. Ding, X. Liang, J. Ma, X. Liu, Y. Zhang, Q. Long, Z. Wen, Z. Teng, L. Jiang, G. Liu, Sono-triggered biomimetically nanoantibiotics mediate precise sequential therapy of MRSA-induced lung infection, *Adv. Mater.* 36 (2024) 2403612, <https://doi.org/10.1002/adma.202403612>.
- [42] J. Yang, X. Zhang, Y. Fu, G. Li, W. Xie, X. Wang, Dual antibiotics-based pH-responsive carrier-free nanodrug against bacterial mixed infection for accelerated wound healing, *Adv. Healthc. Mater.* 14 (2025) e01845, <https://doi.org/10.1002/adhm.202501845>.
- [43] X. Hu, D. Li, H. Li, Y. Piao, H. Wan, T. Zhou, M. Karimi, X. Zhao, Y. Li, L. Shi, Y. Liu, Reaction-induced self-assembly of polymyxin mitigates cytotoxicity and reverses drug resistance, *Adv. Mater.* 36 (2024) 2406156, <https://doi.org/10.1002/adma.202406156>.
- [44] L.-H. Liu, X.-Z. Zhang, Carrier-free nanomedicines for cancer treatment, *Prog. Mater. Sci.* 125 (2022) 100919, <https://doi.org/10.1016/j.pmatsci.2021.100919>.
- [45] H. Bera, C. Zhao, X. Tian, D. Cun, M. Yang, Mannose-decorated solid-lipid nanoparticles for alveolar macrophage targeted delivery of rifampicin, *Pharmaceutics* 16 (2024) 429–440, <https://doi.org/10.3390/pharmaceutics16030429>.
- [46] X. Yang, B. Xie, H. Peng, G. Shi, B. Sreenivas, J. Guo, C. Wang, Y. He, Eradicating intracellular MRSA via targeted delivery of lysostaphin and vancomycin with mannose-modified exosomes, *J. Control. Release* 329 (2021) 454–467, <https://doi.org/10.1016/j.jconrel.2020.11.045>.
- [47] J. Gan, Y. Dou, Y. Li, Z. Wang, L. Wang, S. Liu, Q. Li, H. Yu, C. Liu, C. Han, Z. Huang, J. Zhang, C. Wang, L. Dong, Producing anti-inflammatory macrophages by nanoparticle-triggered clustering of mannose receptors, *Biomaterials* 178 (2018) 95–108, <https://doi.org/10.1016/j.biomaterials.2018.06.015>.
- [48] Y. Wang, V. Ukwattage, Y. Xiong, G.K. Such, Advancing endosomal escape of polymeric nanoparticles: towards improved intracellular delivery, *Mater. Horiz.* 12 (2025) 3622–3632, <https://doi.org/10.1039/D4MH01781A>.
- [49] A. Ahmad, J.M. Khan, B.A. Paray, K. Rashid, A. Parvez, Endolysosomal trapping of therapeutics and endosomal escape strategies, *Drug. Discov. Today* 29 (2024) 104070, <https://doi.org/10.1016/j.drudis.2024.104070>.
- [50] Y. Huang, L. Mu, X. Zhao, Y. Han, B. Guo, Bacterial growth-induced tobramycin smart release self-healing hydrogel for pseudomonas aeruginosa-infected burn wound healing, *ACS Nano* 16 (2022) 13022–13036, <https://doi.org/10.1021/acsnano.2c05557>.
- [51] W.-H. Zhang, J. Wu, L. Weng, H. Zhang, J. Zhang, A. Wu, An improved phenol-sulfuric acid method for the determination of carbohydrates in the presence of persulfate, *Carbohydr. Polym.* 227 (2020) 115332, <https://doi.org/10.1016/j.carbpol.2019.115332>.
- [52] C. Ma, S. Chen, C. Wang, W. Wang, W. Li, H. Feng, Z. Zhao, Defect engineering of Cu₂-xGaO/PDINH nanomaterial for significantly improved photocatalytic antibacterial activities, *Nano Today* 56 (2024) 102230, <https://doi.org/10.1016/j.nantod.2024.102230>.
- [53] L.-X. Yan, L.-J. Chen, X. Zhao, X.-P. Yan, pH switchable nanoplatfor for in vivo persistent luminescence imaging and precise photothermal therapy of bacterial infection, *Adv. Funct. Mater.* 30 (2020) 1909042, <https://doi.org/10.1002/adfm.201909042>.
- [54] D. Zhao, W. Feng, X. Kang, H. Li, F. Liu, W. Zheng, G. Li, X. Wang, Dual-targeted poly(amino acid) nanoparticles deliver drug combinations on-site: an intracellular synergistic strategy to eliminate intracellular bacteria, *J. Mater. Chem. B* 11 (2023) 2958–2971, <https://doi.org/10.1039/D3TB00125C>.
- [55] B. Peng, Y. Su, H. Li, Y. Han, C. Guo, Y. Tian, X. Peng, Exogenous alanine and/or glucose plus kanamycin kills antibiotic-resistant bacteria, *Cell. Metab.* 21 (2015) 249–262, <https://doi.org/10.1016/j.cmet.2015.01.008>.
- [56] K.R. Allison, M.P. Brynildsen, J.J. Collins, Metabolite-enabled eradication of bacterial persisters by aminoglycosides, *Nature* 473 (2011) 216–220, <https://doi.org/10.1038/nature10069>.
- [57] C.M. Webster, M. Shepherd, A mini-review: Environmental and metabolic factors affecting aminoglycoside efficacy, *World J. Microbiol. Biotechnol.* 39 (2022) 7, <https://doi.org/10.1007/s11274-022-03445-8>.
- [58] P. Zou, J. Liu, X. Li, M. Yaseen, J. Yao, L. Liu, L. Luo, H. Wang, X. Shi, Z. Li, T. Sun, Y. Gao, C. Gao, L.-L. Li, A membrane curvature modulated lipopeptide to broadly combat multidrug-resistant bacterial pneumonia with low resistance risk, *ACS Nano* 16 (2022) 20545–20558, <https://doi.org/10.1021/acsnano.2c07251>.
- [59] Y. Li, Y. Li, H. Chen, Y. Chen, Y. Ni, J. Ji, K. Wang, Q. Jin, Self-assembled copper-amino acid nanoleaves for targeted treatment of deep-seated bacterial infections via chemodynamic therapy and cuproptosis-like death, *Biomaterials* 325 (2026) 123566, <https://doi.org/10.1016/j.biomaterials.2025.123566>.
- [60] M. Liu, H. Huang, K. Wang, D. Xu, Q. Wan, J. Tian, Q. Huang, F. Deng, X. Zhang, Y. Wei, Fabrication and biological imaging application of AIE-active luminescent starch based nanoprobes, *Carbohydr. Polym.* 142 (2016) 38–44, <https://doi.org/10.1016/j.carbpol.2016.01.030>.
- [61] Z. Liu, Q. Wang, Z. Zhu, M. Liu, X. Zhao, W.-H. Zhu, AIE-based nanoaggregate tracker: high-fidelity visualization of lysosomal movement and drug-escaping processes, *Chem. Sci.* 11 (2020) 12755–12763, <https://doi.org/10.1039/D0SC04156D>.
- [62] H. Weavers, I.R. Evans, P. Martin, W. Wood, Corpse engulfment generates a molecular memory that primes the macrophage inflammatory response, *Cell* 165 (2016) 1658–1671, <https://doi.org/10.1016/j.cell.2016.04.049>.
- [63] H. Huang, Y. Xie, J. Zhong, Z. Fu, P. Wu, X. Chen, Z. Xiao, J. Yuan, X. Shi, D. Liang, Antimicrobial peptides loaded collagen nanosheets with enhanced antibacterial activity, corneal wound healing and M1 macrophage polarization in bacterial keratitis, *Compos. Part. B* 275 (2024) 111283, <https://doi.org/10.1016/j.compositesb.2024.111283>.
- [64] X. Yang, G. Shi, Z. Lin, Y. Qiu, F. Liu, K. Hu, J. Guo, H. Peng, Y. He, Pathogen-targeting biomineralized bacterial outer membrane vesicles for eradicating both intracellular and extracellular *Staphylococcus aureus*, *J. Control. Release* 382 (2025) 113702, <https://doi.org/10.1016/j.jconrel.2025.113702>.
- [65] H. Tang, W. Chu, J. Xiong, H. Wu, L. Cheng, L. Cheng, J. Luo, H. Yin, J. Li, J. Li, J. Yang, J. Li, Seeking cells, targeting bacteria: a cascade-targeting bacteria-responsive nanosystem for combating intracellular bacterial infections, *Small* 20 (2024) 2311967, <https://doi.org/10.1002/smll.202311967>.
- [66] P. Liu, J. Wu, X. Pei, Z. Cui, J. Jiang, B. Song, B.P. Binks, Recyclable surfactant containing a dynamic covalent bond and relevant smart emulsions, *Green. Chem.* 24 (2022) 7612–7621, <https://doi.org/10.1039/D2GC01977A>.
- [67] X. Kang, X. Yang, F. Bu, W. Feng, F. Liu, W. Xie, G. Li, X. Wang, GSH/pH cascade-responsive nanoparticles eliminate methicillin-resistant *Staphylococcus aureus* biofilm via synergistic photo-chemo therapy, *ACS Appl. Mater. Interfaces* 16 (2024) 3202–3214, <https://doi.org/10.1021/acsaami.3c17198>.