Antibacterial Nanomaterials

Mechanisms, Impacts on Antimicrobial Resistance and Design Principles

Xie, Maomao; Gao, Meng; Yun, Yang; Malmsten, Martin; Rotello, Vincent M. M.; Zboril, Radek; Akhavan, Omid; Kraskouski, Aliaksandr; Amalraj, John; Cai, Xiaoming; Lu, Jianmei; Zheng, Huizhen; Li, Ruibin

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Antibacterial Nanomaterials: Mechanisms, Impacts on Antimicrobial Resistance and Design Principles

Maomao Xie1#, Meng Gao1#, Yang Yun2#, Martin Malmsten3,4, Vincent M. Rotello5, Radek Zboril6,7, Omid Akhavan8, Aliaksandr Kraskouski9, John Amalraj10, Xiaoming Cai11, Jianmei Lu12, Huizhen Zheng1#, Ruibin Li1*

1State Key Laboratory of Radiation Medicine and Protection, School for Radiological and Interdisciplinary Sciences (RAD-X), Collaborative Innovation Center of Radiological Medicine of Jiangsu Higher Education Institutions, Suzhou Medical College, Soochow University, Suzhou, 215123, Jiangsu, China

2College of Environmental & Resource Sciences, Shanxi University, Taiyuan, 030006, Shanxi, China

3Department of Pharmacy, University of Copenhagen, DK-2100 Copenhagen, Denmark

4Department of Physical Chemistry 1, University of Lund, SE-22100 Lund, Sweden

5Department of Chemistry, University of Massachusetts Amherst, 710 N. Pleasant St., Amherst, USA

6Regional Center of Advanced Technologies and Materials, Czech Advanced Technology and Research Institute (CATRIN), Palacký University Olomouc, Šlechtitelů 241/27, Olomouc, 783 71 Czech Republic

7Nanotechnology Center, Center of Energy and Environmental Technologies, VŠB-Technical University of Ostrava, 17. listopadu 2172/15, Ostrava-Poruba, 708 00 Czech Republic

8Condensed Matter National Laboratory, P.O. Box 1956838861, Tehran, Iran

9Department of Physicochemistry of Thin Film Materials, Institute of Chemistry of New Materials of NAS of Belarus, 36 F. Skaryna Str., 220141 Minsk, Belarus

10Laboratory of Materials Science, Instituto de Quimica de Recursos Naturales, Universidad de Talca, P.O. Box 747 Talca, Chile

11School of Public Health, Suzhou Medical College, Soochow University, Suzhou, Jiangsu 215123, China

12College of Chemistry, Chemical Engineering and Materials Science, National Center for International Research on Intelligent Nano-Materials and Detection Technology in Environmental Protection, Soochow University, Suzhou, 215123, China

# Contributed equally

*Corresponding authors:

Dr. Ruibin Li & Dr. Huizhen Zheng

liruibin@suda.edu.cn & hzzheng@suda.edu.cn
Abstract

Antimicrobial resistance (AMR) is one of the biggest threats to the environment and health. AMR rapidly invalidates conventional antibiotics, and antimicrobial nanomaterials have been increasingly explored as alternatives. Interestingly, several antimicrobial nanomaterials show AMR-independent antimicrobial effects without detectable new resistance and have therefore been suggested to prevent AMR evolution. In contrast, some are found to trigger the evolution of AMR. Given these seemingly conflicting findings, a timely discussion of the two faces of antimicrobial nanomaterials is urgently needed. This review systematically compares the killing mechanisms and structure-activity relationships of antibiotics and antimicrobial nanomaterials. We then focus on nano-microbe interactions to elucidate the impacts of molecular initiating events on AMR evolution. Finally, we provide an outlook on future antimicrobial nanomaterials and propose design principles for the prevention of AMR evolution.

Keywords: antimicrobial resistance, antibacterial nanomaterials, nano-bio interaction, killing mechanism, structure-activity relationship
1 Introduction

Antimicrobial resistance (AMR) is regarded as one of the worst threats to global public health.[1] Recently, a report in the Lancet estimated that approximately 1.27 million deaths in 2019 could be attributed to AMR bacteria, involving six leading pathogens, i.e., *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*. AMR is increasingly spreading and globally evolving due to intrinsic gene mutations and/or phenotype changes under the survival pressure of abused antibiotics[3] or horizontal gene transfer (HGT) of antibiotic resistance genes (ARGs), which can persist for up to six months in patients post-antibiotic treatment, thus resulting in “antibiotic scarring”. Although many AMR mutations are associated with considerable fitness costs, such as i) the reduction of bacterial growth, virulence or transmission and ii) suppressed stability of resistance and impotent competitiveness with commensals and/or other flora species,[5] a recent WHO report estimates that AMR-based infections may cause 350 million deaths by 2050 if unchecked.[6] Resistant bacteria (a.k.a. superbugs) have been detected in many regions and countries. The first resistant strain, penicillin-resistant *Staphylococcus* was identified in 1940.[7] After that, tetracycline-resistant *Shigella*, methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus*, levofloxacin-resistant *Pneumococcus*, and imipenem-resistant *Enterobacteriaceae* were consecutively identified.[7-8] Recently, excessive consumption of various types of antibiotics during the COVID-19 pandemic[9] as well as their significant leakage into the environment[10] have resulted in further unwanted antibiotic exposure around the world and exacerbated AMR evolution. In stark contrast to this alarming development, there have been no new classes of clinical antibiotics for the last 40 years except for the discovery of two narrow-spectrum drugs, daptomycin and linezolid.[11] Strikingly, it takes only 12 months for *Enterococcus faecium* to adapt to daptomycin.[12] In 2015, Liu *et al.* discovered the emergence of plasmid-mediated mobile colistin resistance to polymyxins, signifying the breach of the last group of antibiotics.[13] Even worse, most pharmaceutical enterprises have greatly reduced their investment in the research and development of new antibiotics. As a result, we are...
stepping into a “post-antibiotic” era. While the WHO and other health organizations and professionals appeal to the public for reducing the abuse of antibiotics, increasing attention in academic research has therefore developed during the last few years to explore alternatives to antibiotics.

The rapid development of nanotechnology provides promising strategies to combat resistant bacteria.\textsuperscript{[14]} Compared to conventional antibiotics, nanomaterials allow rational engineering designs, such as size control, surface modification, crystalloid change and stimuli-responsive functionalization, to acquire distinct interactions with bacterial cells.\textsuperscript{[14b]} These interactions often result in unique killing mechanisms and extraordinary antimicrobial features, such as broad-spectrum activity,\textsuperscript{[15]} long-lasting and resistance-independent antimicrobial effects.\textsuperscript{[16]} For instance, graphene oxide (GO) nanosheets are reported to show broad-spectrum antibacterial effects in both Gram-positive and Gram-negative multidrug resistant (MDR) bacteria due to physical membrane disruption effects.\textsuperscript{[17]} Boron nitride (BN) nanosheets were found to exhibit strong antibacterial activity against five resistant bacterial strains by a cell division arrestment mechanism.\textsuperscript{[16b]} In addition to the superior antibacterial effects of these nanomaterials toward AMR cells, their long-term use did not trigger detectable secondary resistance, \textit{i.e.}, these antimicrobial nanomaterials were concluded to prevent AMR evolution. However, some recent studies have demonstrated that bacteria, including \textit{E. coli} and \textit{P. aeruginosa}, can develop resistance to antimicrobial nanomaterials (\textit{e.g.}, Ag NPs)\textsuperscript{[18]} as well as cross-resistance to antibiotics due to bacterial adaptation to nanomaterials (\textit{e.g.}, ZnO NPs or polystyrene nanoplastics\textsuperscript{[19]}). Briefly, evolutionary trajectories under nonlethal selective pressure of nanomaterials may produce distinct resistance mechanisms, such as efflux system mutations,\textsuperscript{[18b]} outer membrane porin (Omp) family suppression,\textsuperscript{[20]} HGT,\textsuperscript{[19b]} flagellin production,\textsuperscript{[18a]} and cell envelope remodeling.\textsuperscript{[21]} Considering these seemingly conflicting reports, as well as the extensive use of nanomaterials in a wide range of products,\textsuperscript{[22]} a timely discussion of the two faces of antimicrobial nanotechnology in AMR evolution is highly needed to reduce the risk of AMR spreading.
In this review, we systematically compare the killing mechanisms and structure-activity relationships (SARs) of traditional antibiotics with nanomaterials. Due to the two-face impacts of nanotechnology on AMR evolution, we further highlight nano-microbe interactions to elucidate the impacts of molecular initiating events (MIEs) on AMR evolution. Based on this, design principles of antimicrobial nanomaterials are proposed to prevent AMR evolution. Finally, we discuss the prospects of antimicrobial nanotechnologies in future uses.

2 Comparisons between Antibiotics and Antimicrobial Nanomaterials

2.1 Uptake and Distribution in Bacteria

The size of antimicrobial nanomaterials generally lies in the range of 1-100 nm, while conventional antibiotics are small organic molecules with molecular weights < 1000 Da (diameter < 1 nm). Due to this difference in size, these two classes of compounds exhibit different interactions with bacteria. Most antibiotics, such as lincomycin, clindamycin, carbapenems, β-lactams, quinolones and aminoglycosides, can permeate into the cytoplasm, either by transmembrane diffusion or porin-mediated passive diffusion, such as OmpF/OmpC porins.[23] Because of the size limitation (≈ 10 Å), the porin channels can only transport small drugs (Mw < 600 Da).[24] In a few cases, some specific channels with strong affinity sites for certain molecular structures allow the transport of antibiotics, such as maltose-specific channels (LamB) and carboxylate-specific channels (OprD), for carbapenem uptake.[25]

In contrast to the clear cell internalization pathways of antibiotics, nano-microbe interactions are less well defined. Substantial evidence has shown that most nanomaterials are incapable of cytoplasmic internalization but mainly interact with the cell wall/membrane components (Table 1) by destabilization, oxidative damage, mechanical disruption, and thermal effects.[17, 26] For instance, GOs and their nanocomposites were disclosed to disrupt the integrity of MDR *E. coli* cells by interacting with the lipid membrane or peptidoglycan (PGN) layer.[18c, 27] Quasi-
spherical Au NPs with a diameter of 100 nm were evidenced to show a high affinity to the bacterial membrane, causing lethal stretching or indentations.\cite{28} Coincidentally, this interaction is similar to some newly discovered antibiotics, such as teixobactin targeting lipid II\cite{29} and darobactin stabilizing surface protein BamA.\cite{26a}

Given that endocytosis is the dictating mechanism for the internalization of nanomaterials by mammalian cells,\cite{30} the poor cytoplasmic internalization of such materials in bacterial cells may be attributed to the deficiency of endocytosis pathways.\cite{17} However, some researchers have reported that small nanomaterials can be internalized into bacterial cells (Table 1). For instance, several noble metal NPs, such as Pd nanocrystals\cite{31} and Au nanoclusters\cite{32}, were identified in the cytoplasm by transmission electron microscopy (TEM) observation and element mapping. Linklater et al. demonstrated that ultrasmall Au nanoclusters with a 2 nm diameter could freely translocate across the bacterial membrane to trigger devastating cytoplasmic damage.\cite{33} Notably, internalized nanomaterials display small sizes of 1-10 nm, and some nanocrystals,\cite{31} nanoclusters,\cite{32} quantum dots,\cite{34} and metal-organic frameworks (MOFs)\cite{35} of that size range show excellent killing efficiency. However, small metallic NPs usually have a large active surface and may release ions in biological media. The released metal ions may be taken into bacterial cells and precipitate into new NPs.\cite{36} This transformation behavior of metallic NPs is often less considered in biodistribution studies.

### 2.2 Antimicrobial Mechanisms

#### 2.2.1 Antimicrobial Pathways of Antibiotics

Up to 145 antibiotics have been approved in the clinic during the past century.\cite{37} These compounds have specific targets and display good differentiation capability between bacteria and eukaryotic cells. According to the biological functions of their targets, the antimicrobial mechanisms are divided into five classes (Figure 1), including i) inhibition of cell wall synthesis; ii) depolarization of the cell membrane; iii) inhibition of protein synthesis; iv) inhibition of nucleic acid synthesis; and v) inhibition of...
metabolic pathways. The antimicrobial mechanism of cell wall inhibition is often reported in β-lactams (e.g., cephalosporins, ceftobiprole, and ceftaroline) by covalent binding of penicillin-binding proteins (PBPs) for blockage of PGN biosynthesis (Figure 1a).[38] The antimicrobial effects of peptides (e.g., linearmycins,[39] polymyxin,[40] and cecropin[41]) are attributed to the cell membrane depolarization mechanism by binding with lipopolysaccharide (LPS) and/or phospholipids (Figure 1b). Most clinical antibiotics, such as macrolides, aminoglycosides, tetracyclines, lincosamides and chloramphenicols, exert their antimicrobial effects to block protein synthesis on ribosomes (Figure 1c) by targeting three functional centers of the ribosome: i) the decoding region at the 30S subunit, ii) the peptidyl transferase center at the 50S subunit, and iii) the ribosome exit tunnel for the passage of nascent polypeptide chains.[42] Quinolones are reported to prevent DNA unwinding and duplication by inhibiting critical enzymes (DNA gyrase and topoisomerase IV) in nucleic acid synthesis (Figure 1d).[43] Metabolic pathway inhibition is reported in some antibiotics that can either take over a metabolic substrate to bind with its target or boost the production of toxic byproducts (Figure 1e).[44]
Figure 1. Antimicrobial mechanisms of antibiotics

Antibiotics may prevent bacterial growth by a) inhibiting cell wall synthesis; b) depolarizing the cell membrane; c) inhibiting protein synthesis; d) inhibiting nucleic acid synthesis; and e) inhibiting metabolic pathways. β-lactams can bind with PBPs to block PGN biosynthesis. Antimicrobial peptides often interact with LPS and/or phospholipids to depolarize the cell membrane. Antibiotics targeting ribosomes can prevent protein synthesis and are widely used in clinics. Inhibition of enzymes in nucleic acid synthesis results in failure of DNA unwinding and duplication. Only a few antibiotics (e.g., sulfonamides) have been reported to inhibit the conversion of para-aminobenzoic acid (PABA) into dihydrofolic acid (DHFA) for blockage of bacterial proliferation.

2.2.2 Antimicrobial Pathways of Antibacterial Nanomaterials

During the past decade, there have been more than 400 reported antimicrobial nanomaterials from 128 countries. Due to the limitations of nanotechnology in standardization, a number of nanobiology studies have low reproducibility.\[^{[45]}\] We
analyzed the publications to select antimicrobial nanomaterials for further discussion. As a result, five classes of antimicrobial nanomaterials were widely reported, including metallic NPs (e.g., Ag, Cu, Au, ZnO, La_2O_3, CeO_2, V_2O_5, etc.), carbonaceous nanomaterials (e.g., GO, graphene, carbon nanotube (CNT)), borides (e.g., BN), nanosized polymers (e.g., polycarbonate) and nanocomposites (e.g., La_2O_3/Ag-GO). Ag NPs, Au clusters, ZnO and CuO nanomaterials, displayed high antimicrobial efficiency with minimum inhibitory concentrations (MICs) as low as those of antibiotics, while other antimicrobial nanomaterials displayed relatively low efficiency compared to antibiotics. BN nanosheets and polysaccharide-capped Ag NPs showed good selectivity among different bacterial strains or between eukaryotes and prokaryotes. In contrast, most antimicrobial nanomaterials showed similar killing effects in bacterial and mammalian cells. There have been six identified antimicrobial mechanisms according to MIEs, including membrane destruction, disruption of the electron transport chain, catalytic killing, cell division arrest, prolonged ionic killing and nanoparticle aggregation mediated cell trapping.

**Membrane Destruction.** Membrane disruption is one of the most important antimicrobial mechanisms of nanomaterials, including physical destruction (e.g., shape-mediated cutting effect, particle-mediated membrane destabilization, bubble-mediated destruction effect, lipid extraction, etc.) and chemical destruction (e.g., oxidation or dephosphorylation effect). Carbonaceous nanomaterials, such as CNTs\(^\text{[46]}\) and graphene derivatives\(^\text{[27b, 47]}\), often physically interact with bacterial cell walls and/or membranes to destroy these protective covers and result in the leakage of cytoplasmic components, although such effects may interplay with oxidative and other effects. GO was extensively reported to cause bacterial membrane damage via basal plane or sharp edge destruction pathways by inserting, wrapping, or trapping into the cell membrane (Figure 2a).\(^\text{[27b, 47]}\) Lu et al. studied the antibacterial effects of GO composite films with different exposure orientations, indicating that vertically oriented nanosheets exhibited enhanced antibacterial activity in comparison to both random and horizontal orientations.\(^\text{[48]}\) Del Valle et al. demonstrated that a mechanical force of approximately
20 nN was capable of breaking *E. coli* cell walls.\(^{[49]}\) Hence, 1D or 2D nanomaterials with sharp edges or amphiphilic structures may generate such mechanical force and act as a nano-knife or cutter to damage bacterial walls/membranes.\(^{[50]}\) Spiky or “virus-like” nanomaterials may also have strong binding affinity with the membrane to destabilize it.\(^{[51]}\) Physical destruction may involve one or more types of molecular interactions, *e.g.*, van der Waals, hydrophobic and electrostatic forces.\(^{[27b]}\) Nederberg *et al.* reported that amphiphilic polycarbonate could assemble into cationic micellar nanostructures for cracking the membrane in MRSA and fungi by steric hindrance, hydrogen binding and electrostatic interaction with the cell wall/membrane.\(^{[52]}\) Tu *et al.* conducted molecular dynamics (MD) simulations to clarify the lipid extraction processes of graphene (Figure 2a) and found that van der Waals interactions and hydrophobic interactions contribute to lipid extraction from the membrane by graphene nanosheets, resulting in *E. coli* cell collapse.\(^{[27b]}\) However, there is a lack of convincing experimental data to rank the contributions of each interaction force to the antimicrobial effect of nanomaterials. In addition to these direct interactions, some nanomaterials (*e.g.*, graphene derivatives) may assist the generation\(^{[53]}\) and long-term storage\(^{[54]}\) of micro/nanobubbles, which could induce cell wall/membrane destruction and/or interruption in bacterial respiration (Figure 2a).\(^{[55]}\)

Unlike physical interactions, chemical destructions often involve the molecular skeleton changes of components in cell envelopes by reacting with nanomaterials. For example, La\(_2\)O\(_3\) NPs were reported to react with phospholipids to transform into LaPO\(_4\) on Gram-negative bacterial cell membranes, resulting in dephosphorylation of phospholipids and an unordered membrane configuration as well as increments in membrane fluidity and permeability (Figure 2a).\(^{[56]}\) Nanomaterials with this killing mechanism often show antimicrobial activity in diverse bacterial strains but have poor selectivity to differentiate bacterial and mammalian cells.

**Disruption of the Electron Transport Chain.** Redox reactions pervade almost all biological processes in living microorganisms\(^{[57]}\) and display a broad redox potential
range of -4.12 to +4.84 eV.\[58\] Nanomaterials with conduction band energies falling into this cellular redox potential may prevent bacterial growth by interfering with the biological electron transport chain. Li et al. prepared a semiconductor-to-metal phase-changed thin film by doping tungsten with VO\(_2\) to capture electrons from the transmembrane protein complex of the respiratory chain, thereby causing bacterial oxidative stress and energy deprivation (Figure 2b).\[59\] Alkaline magnesium oxide films sputtered on polyetheretherketone polymer and zirconia ceramic implants impeded bacterial respiration action, disrupted ATP synthesis and blocked energy metabolism by a similar mechanism.\[60\] In addition to metallic nanomaterials, CNTs with metallic-like characteristics allow ballistic electron transport between the tube surface and bacterial membrane, proteins, lipids and DNA, resulting in dysfunction of membrane proton transport and chemical oxidation of biomacromolecules.\[61\] However, these antimicrobial nanomaterials often have high oxidative reactivity and may become invalid in complex biological contexts (e.g., blood) by reacting with reductive species.

**Catalytic Killing.** The catalytic killing mechanism has gained increasing attention in recent years due to its broad, potent, and persistent bactericidal activities. Recently, a growing number of inorganic NPs (a.k.a. nanozymes) have been discovered to exhibit enzyme-like activities. For example, Fe\(_3\)O\(_4\),\[62\] CuO,\[63\] ZnO,\[64\] V\(_2\)O\(_5\),\[65\] CeO\(_2\),\[66\] GO,\[67\] CNTs,\[68\] doped graphene\[69\] and SnSe\[70\] have all been reported to show peroxidase-, superoxide dismutase-, peroxidase-, oxidase-, catalase- or dehydrogenase-like activities. These nanozymes may participate in biological processes to disturb bacterial growth and metabolism (Figure 2c). For instance, CeO\(_2\)-x nanorods with lower aspect ratios containing higher Ce\(^{3+}\) sites could exhibit higher haloperoxidase-like activity and remarkable bactericidal activity in *E. coli*.\[71\] Spherical CeO\(_2\) synthesized in phosphate-free solutions was able to mimic the activity of phosphatase and efficiently catalyze the hydrolysis of P-O bonds of LPS,\[72\] which could potentially prevent the growth of Gram-negative bacteria. Compared to natural enzymes, nanozymes have limited catalytic selectivity toward different substrates, which may lead to poor killing selectivity. The identification of nanozymes mimicking a specific
enzyme in certain bacterial strains is likely a promising strategy to overcome this limitation. In addition to direct catalysis, nanomaterials may catalyze specific substances in extracellular media to generate toxic species. Thus, graphene quantum dots (GQDs) with peroxidase-like activity,[73] V2O5 nanowires with haloperoxidase-like activity[74] and nickel disulfide nanozymes with both peroxide- and glutathione peroxidase-like activities[75] can generate •OH, 1O2 and/or HOBr species in the presence of H2O2, O2, and/or Br− to kill bacteria (Figure 2c). In addition, photoactive nanomaterials, including carbon dots, graphitic carbon nitride nanosheets (g-C3N4), transition metal oxides, and noble metal NPs, can catalyze diverse oxidative species (e.g., 1O2, •OH) under light illumination for photodynamic elimination of bacteria.[26b, 76] These nanomaterials are rarely used in clinics while can be widely applied in the environment to prevent biofilm formation and display long-lasting antimicrobial effects.

**Cell Division Arrest.** Bacterial cell division is a complex and dynamic process that begins with FtsZ polymerization to form a Z ring and ends with Z ring constriction/degradation, involving cell wall synthesis and hydrolysis.[77] In detail, FtsZ, as a cytoskeletal protein with a GTP-binding motif (GGGTGS/TG), displays GTPase activity that can hydrolyze GTP into GDP. The FtsZ-GDP monomers self-assemble into filaments (Z ring) by incorporating a nucleotide-binding site into the T7-loop region of polymers.[78] The mature Z ring recruits more division proteins for the formation of the septum. After that, the Z rings constrict to separate two newborn daughter cells. Interestingly, some nanomaterials were found to display antimicrobial effects by preventing Z ring formation, localization or degradation. For example, polysaccharide-capped Ag NPs with an average diameter of 20-40 nm were able to block bacterial cell growth by inhibiting the expression and oscillation of the FtsZ-FtsA complex (Figure 2d).[79] Thiol-stabilized Cu NPs were found to destabilize FtsZ and FtsI and block Z ring formation to prevent bacterial cell division, while they exhibited biocompatibility on murine macrophages.[80] Ag and Cu NPs are reported to prevent Z ring formation by affecting the expression and positioning of bacterial division proteins (FtsZ, FtsA, FtsI) or destabilizing them. However, the chemical basis for this phenomenon is unclear.
addition to the disruption of Z ring formation, Pan et al. showed that BN nanosheets impaired Z-ring constriction in MDR E. coli. As a result, bacterial cells were arrested in the binary fission process and failed to divide into two daughter cells after exposure to BN nanosheets. Biotin labeling of the surface proteome was performed to identify their targets, suggesting that BN could interact with multiple surface proteins (e.g., FtsP, EnvC, TolB) in cell division, resulting in cell duplication disorder (Figure 2d).\textsuperscript{116b} MD simulations suggested that some amino acids (e.g., alanine, serine, phenylalanine, glutamine, and glycine) with uncharged or hydrophobic side chains could strongly bind with BN nanosheets. The strong binding affinity is attributed to multiple synergistic interactions, including steric hindrance of planar shapes and $\pi-\pi$ and CH–$\pi$ interactions.

![Figure 2](https://example.com/figure2.png)

**Figure 2. Killing mechanisms of antimicrobial nanomaterials**

The antimicrobial mechanisms of nanomaterials are determined by their MIEs, involving a) membrane destruction by physical/chemical interactions, b) electron transport chain disruption by metallic or semiconductor nanomaterials, c) catalytic killing by mimicking enzymes for direct destruction of critical biomolecules or ROS-
mediated damage, d) cell division arrest by blockage of Z ring formation/degradation, 

e) prolonged ionic killing by inactivating cellular enzymes, disrupting respiratory 
processes and elevating intracellular ROS and f) NP aggregation-mediated cell trapping 
by noncovalent interactions.

**Prolonged Ionic Killing.** Silver, gold, copper, nickel, iron, manganese, zinc, mercury, 
and cadmium have all been popular bactericidal elements since ancient times. These 
metals could take effect via inactivation of cellular enzymes, destruction of the electron 
transport chain in the respiratory process and eruption of intracellular ROS (Figure 
2e).[81] Although metal ions have high bactericidal performance, their applications are 
limited due to their first-pass effects and safety concerns. Engineering these metal 
elements into nanosized objects may promisingly overcome those limitations by 
prolonged ion release.[82] Ag⁺, a classical bactericidal ion, can be slowly released from 
Ag NPs,[83] causing lipid peroxidation, GSH depletion and bacterial DNA degradation 
(Figure 2e).[84] However, some reports argue that the killing effects of metallic 
nanomaterials are largely dependent on particle-mediated membrane destabilization 
rather than ion release.[85] For instance, Hu et al. found that the rate of lipid flip-flop 
increased with Ag NP concentration increasing,[86] and Vishnupriya et al. used Raman 
spectroscopy to disclose that Ag NPs could penetrate into bacterial cells and interact 
with the exocyclic nitrogen of adenine, guanine and cytosine bases, leading to DNA 
damage.[85a] Recently, an increasing number of studies have indicated that both ion and 
particle effects are involved in the bactericidal activity of metallic nanomaterials.[87] 
Notably, released metal ions have been reported to induce resistance in bacteria. 
Nanomaterials with this killing mechanism are unavailable to prevent AMR spread.

**Nanoparticle Aggregation Mediated Cell Trapping.** Cell culture ingredients (e.g., 
bovine serum albumin (BSA)[88]), antioxidant compounds (e.g., vitamin C[89]), and/or 
glycolysis of bacteria[90] may result in the reduction of oxygen-containing functional 
groups on the NP surface, destroying colloidal stability and causing NP aggregation. 
This phase change will trap bacteria present in the suspension or culture media and 
consequently inactivate them[91]. Illustrating this, LL-37 peptide-coated laponite NPs
could induce the flocculation of Gram-negative bacteria by laponite-LPS interactions, thereby localizing infection and inflammation (Figure 2f). This strategy has also been exploited in challenges against new generations of viruses. In contrast to others, it should be combined with other killing mechanisms to acquire the expected antimicrobial activity.

2.3 Structure-activity Relationships

2.3.1 Rules to Tune the Molecular Properties of Antibiotics

Most antibiotics have specific core structures (e.g., β-lactam ring, 4-quinolone, macrocyclic lactone ring and tetracene) to bind with their targets. Candidate antibiotics are often designed to follow Lipinski’s rule: i) less than 5 hydrogen bond donors, ii) less than 10 hydrogen bond acceptors, iii) molecular mass < 500 Da, and iv) octanol-water partition coefficient < 5. This rule well describes the molecular properties of drug candidates that may impact the attrition rates in clinical trials and the chance of launch. However, only 50% of orally administered compounds obey it. Some natural products, such as macrolides and peptides, break the filter thresholds used in Lipinski’s rule. Therefore, attempts have been made to improve the performance of antibiotics by tuning their molecular charge, lipophilicity, and configurations.

2.3.2 Physicochemical Properties Dictating the Antimicrobial Efficiency of Nanomaterials

Compared to antibiotics, antimicrobial nanomaterials often have highly variable physicochemical properties, such as size, shape, crystallinity, surface charge, surface defects, and vacancies. These properties could be deliberately tuned to acquire the expected nano-microbe interactions.

**Composition.** Chemical composition plays a decisive role in the antimicrobial effects of metallic nanomaterials, from which released metal ions are often regarded as the dominant factor. In addition to particle-mediated membrane destabilization, many metal ions (e.g., Ag⁺, Zn²⁺, Cu²⁺, Mn⁴⁺, and Ni³⁺) are known to display
antimicrobial effects in a broad spectrum of bacteria. Consequently, diverse antimicrobial nanomaterials may be designed by these elements, such as Ag,[100] ZnO,[101] CuO,[102] MnO2,[103] and Ni2O3[104] NPs. In addition, the rational design of metal dopants may alter their electronic and catalytic activities to exert antimicrobial effects. For instance, doping Pt in Au NPs could remarkably enhance their enzyme-like activity to catalyze intracellular ATP production, thereby causing membrane potential collapse and cell death.[105] In addition to transition metals, rare earth NPs are reported to destroy the envelopes of Gram-negative bacteria by dephosphorylation and disrupting membrane structures.[18c, 56] However, the antimicrobial effects of nonmetallic nanomaterials are less affected by the composition, as their morphology, size and surface functionalization can be more important features.[106]

Size. Many NPs, e.g., Ag,[107] ZnO[108] and gold colloids,[109] show size-dependent antimicrobial effects. The antimicrobial effect of 2D nanosheets may be impacted by their planar length and vertical thickness.[17] Most studies demonstrate that the antibacterial effect of GOs was markedly enhanced along with their thickness decrement.[110] The effects of lateral size on the antibacterial activity of GOs are still somewhat controversial. Liu et al. found that large GO nanosheets could fully cover bacterial cells to inhibit their proliferation, whereas small nanosheets had a low antibacterial effect.[111] In contrast, Perreault et al. found that small GO with an average area of 0.01 μm² showed higher antibacterial activities than those at 0.1-0.65 μm², probably because small GO had more surface defects.[112] In addition, Ti3C2Tx MXene nanosheets were found to display lateral size-dependent killing effects. When the size decreased from 497 nm to 196 nm, the photothermal antibacterial activity against MRSA was markedly enhanced.[113] For most antimicrobial nanomaterials, a smaller size is often considered to be favorable for killing bacteria,[114] probably because these particles have larger active surfaces.

Shape/Crystallinity. MD simulations have revealed that shape is crucial for the interaction between NPs and lipid bilayers.[115] For instance, spherical Au NPs display
stronger interactions with bacterial cells than rod-shaped Au NPs.\textsuperscript{116} Precise shape tuning by atomic arrangement, particularly facet orientation, determines the catalytic, redox and ion release performances of nanomaterials.\textsuperscript{117} The strong adsorption and Ag\textsuperscript{+} release synergistically contributed to the higher antibacterial activity of cubic Ag\textsubscript{2}O compared to that of the octahedral material.\textsuperscript{118} Commonly, high-index/energy facets are characterized by higher catalytic oxidation and stronger bioactivity. For example, TiO\textsubscript{2} with high-energy \{100\} facets showed stronger killing effects than TiO\textsubscript{2} with \{101\} facets.\textsuperscript{119} A similar facet-dependent catalytic bactericidal effect has been demonstrated in Pd nanocrystals. Cubic Pd with \{100\} facets has higher oxidase and peroxidase-like activities than octahedral Pd with \{111\} facets and shows a better antibacterial effect against Gram-positive bacteria.\textsuperscript{31} In contrast, octahedral Pd displays stronger antibacterial capability than cubic Pd in Gram-negative bacteria. The different antibacterial effects have been ascribed to the combined actions of facet-dependent penetration behavior and enzyme-like activity in Gram-positive and Gram-negative bacteria.\textsuperscript{31} In another study of cubic and octahedral-shaped Cu\textsubscript{2}O, it was also found that one with an octahedral shape has better activity against \textit{E. coli}.\textsuperscript{120}

\textbf{Surface Ligands.} The surfaces of bacterial cells are negative, so antimicrobial nanomaterials are often deliberately functionalized as cationic surfaces to enhance their interactions with bacteria. For example, polyethyleneimine (PEI)-functionalized Fe\textsubscript{3}O\textsubscript{4} with a $\zeta$ potential of $+23$ mV displayed stronger adhesion and penetration ability to bacterial cells and biofilms than citrate-modified Fe\textsubscript{3}O\textsubscript{4} with a negative charge.\textsuperscript{121} Consistently, compared to the negatively charged Ag NPs with citrate or polyvinylpyrrolidone modification, the branched PEI-coated Ag NPs with $+40$ mV $\zeta$ potential displayed strong interaction with bacteria, displaying excellent bactericidal capability.\textsuperscript{122} However, excessive charge contrasts, such as the higher content of cationic dioleoyltrimethylammonium propane in Au NPs reported by Xiao \textit{et al.}, may lead to robust binding with lipid headgroups, limited bilayer internalization, and poor membrane destabilization.\textsuperscript{123} Since Gram-negative and Gram-positive bacteria display different surface charges,\textsuperscript{124} Pillai \textit{et al.} demonstrated Gram selectivity by regulating
the proportions of mixed ligands on Au NP surface shells for desired polyvalent electrostatic and noncovalent interactions with bacterial cell walls. As PEI and many other cationic surface modifications are toxic, coating NPs with lower toxicity antimicrobial peptides represents an interesting alternative. In addition to surface charge, the hydrophobicity of NPs is often determined by surface ligands. To assess this, Li et al. functionalized positively charged Au NPs with different hydrophobic end groups (e.g., alkyl chains and aromatic groups), revealing that Au NPs bearing hydrophobic \textit{n}-decane with the highest Log P value exhibited the highest antibacterial activity against \textit{E. coli}.

**Surface Defect/Vacancy.** Surface defects/vacancies are closely related to the electronic/catalytic activities of nanomaterials and therefore impact their antimicrobial effects. The oxygen vacancies involved in the structure of semiconductor (e.g., TiO\textsubscript{2}, WO\textsubscript{3}, ZnO) surfaces may capture abundant electrons to interact with absorbed O\textsubscript{2} and facilitate the generation of various oxidative species, including \textit{O}\textsubscript{2}, \textit{O}\textsubscript{2}\textsuperscript{2−} and \textit{O}\textsubscript{4}\textsuperscript{2−}, for bacterial killing. Illustrating this, Hsu \textit{et al.} designed Au-doped BiOI nanosheets to improve the oxidase-like activity for synergetic killing of both sensitive and drug-resistant bacteria by elevating the oxygen vacancy density. In addition, Xu \textit{et al.} found that sulfur vacancies on MoS\textsubscript{2} and WS\textsubscript{2} nanosheets contributed to the production of free radicals and interactions with phospholipids. Apart from anion vacancies (e.g., O, S, C, and N vacancies), a close correlation between cation vacancies (e.g., Zn vacancies in ZnIn\textsubscript{2}S\textsubscript{4} layers), vacancy clusters/pits and photocatalytic behavior has been demonstrated but has rarely been explored to improve the effects of antimicrobial nanomaterials. In addition to metallic nanomaterials, the antimicrobial activity of carbonaceous nanomaterials is determined by their surface defects. For example, smaller or hydrolyzed GO nanosheets typically present more defects and display excellent antibacterial effects. Consistently, CNTs with small diameters and high defect densities are favorable for the production of triplet excited states, thereby yielding a higher level of singlet oxygen for killing bacteria.
**Surface Heterojunctions.** Heterojunctions with unique features are currently used in versatile antibacterial purposes based on light-driven photocatalytic mechanisms.\[133\] Since the antibacterial activity of nanomaterials is a surface phenomenon, the thickness of the heterojunction should be optimized to obtain effective surface reactions.\[134\] In addition, the porosity, hydrophilicity, and chemical stability of heterojunction surfaces are important for long-lasting antibacterial effects. In some cases, the best thickness for one of the components of the heterojunctions is atomic thickness, *i.e.*, the presence of 2D and/or MXene materials.\[135\]

### 2.4 Advantages/Disadvantages of Antimicrobial Compounds and Nanomaterials

Millions of lives have been saved since the first antibiotic discovered in 1942. Almost all clinically approved antibiotics display definite chemical structures and are produced by either chemical synthesis or extraction from microorganisms. Since the physicochemical properties of antibiotics are unique, their scale-up production and purification procedures could be well standardized (Table 1).\[136\] Their bactericidal pathways allow highly efficient and specific killing of bacteria but offer clear mutation targets for bacterial cells to adapt to different antibiotics. After long-term adaptation, bacterial cells have developed mature resistance mechanisms, such as i) inactivating antibiotics by hydrolytic enzymes; ii) modifying the antibiotic target sites; and iii) altering the permeability by increasing efflux pumps or causing porin loss.\[137\] As a result, new antibiotics are supposed to be rapidly tolerated by AMR evolution. Due to the transient bactericidal effects and limited penetration capability in biofilms, disabling biofilm resistance is still refractory to antibiotics (Table 1). As ‘magic bullets’ against infections, antibiotics interact with specific targets to selectively prevent bacterial growth, exhibiting limited side effects *in vivo*. However, it is difficult to control the distribution of antibiotics in infected organs, which may destroy beneficial symbiotic bacterial communities and residue in healthy tissues.\[138\] All these challenges dramatically thwart the development of new antibiotics.
Table 1. Comparisons of antibiotics and antimicrobial nanomaterials

<table>
<thead>
<tr>
<th></th>
<th>Antibiotics</th>
<th>Antimicrobial nanomaterials</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Production</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standardization</td>
<td>●●●</td>
<td>●●  ○</td>
</tr>
<tr>
<td>Purification</td>
<td>●●●</td>
<td>●○  ○</td>
</tr>
<tr>
<td>Scale-up production</td>
<td>●○○</td>
<td>●○  ○</td>
</tr>
<tr>
<td><strong>Bacterial killing</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Killing efficiency</td>
<td>●●●</td>
<td>●●  ○</td>
</tr>
<tr>
<td>Target</td>
<td>Specific</td>
<td>Multiple</td>
</tr>
<tr>
<td>Biodistribution</td>
<td>Intermembrane/intracell</td>
<td>Extracell/intermembrane/intracell</td>
</tr>
<tr>
<td>AMR evolution</td>
<td>●●●</td>
<td>●●  ○</td>
</tr>
<tr>
<td>AMR prevention</td>
<td>●○○</td>
<td>●○  ○</td>
</tr>
<tr>
<td><strong>Biofilm prevention</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Permeability</td>
<td>●○○</td>
<td>●○  ○</td>
</tr>
<tr>
<td>Durability</td>
<td>●○○</td>
<td>●●  ●</td>
</tr>
<tr>
<td>Efficiency</td>
<td>●○○</td>
<td>●○  ○</td>
</tr>
<tr>
<td><strong>In vivo application</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Side effect</td>
<td>●○○</td>
<td>●●  ○</td>
</tr>
<tr>
<td>Selectivity</td>
<td>●○○</td>
<td>●○  ○</td>
</tr>
<tr>
<td>Controllability</td>
<td>●○○</td>
<td>●●  ●</td>
</tr>
</tbody>
</table>

**Note:** The black dots represent the intensity degree. Side effect refers to an adverse effect of antibacterial agents that is in addition to or beyond its desired effect. Controllability refers to the ability of antibacterial agents to be engineered for desired biological behaviors in vivo, such as prolonged blood circulation, specific biodistributions, etc.

In striking contrast, antimicrobial nanomaterials as alternatives have been increasingly explored in the past ten years to overcome the shortcomings of antibiotics. Diverse synthesis methods, including physical grinding/peeling, hydrothermal reaction, chemical vapor deposition, sol-gel reaction, combustion, etc., have been reported to prepare nanomaterials, while the standardization of characterization and purification is challenging, thereby restricting their scale-up production and applications (Table 1).[139]

Currently, several nanomaterials, including Ag, TiO2 and chitosan-coated poly lactic-co-glycolic acid (PLGA) NPs, are in clinical trials against fungal, aspergillosis or candida infections.[140] In terms of killing mechanisms, most antimicrobial nanomaterials interact with either multiple targets or specific conserved bacterial constituents that are hard to modify by gene mutations.[18c] The variable physicochemical properties of nanomaterials provide more opportunities for engineering design,[141] which can confer long-lasting and controllable bactericidal effects and a high capability to prevent/degrade biofilms.[142] These features make
antimicrobial nanomaterials promising candidates to kill resistant bacteria in biofilms and prevent AMR spread. However, bacterial adaptations have also been reported for antimicrobial nanomaterials with specific interaction targets and killing mechanisms, leading to resistance to either nanomaterials or antibiotics. Additionally, some formidable barriers, e.g., low dispersibility, poor selectivity, unknown toxicity, reticuloendothelial system and mucosal layer trapping, biofilm extracellular matrices and macrophage capture, are still challenges in their clinical applications.

3 AMR Evolution in Nano-microbe Interactions
Antimicrobial nanomaterials are often exploited to kill MDR bacteria because they exhibit AMR-independent bactericidal effects. Moreover, the features of antimicrobial nanomaterials, such as diverse killing mechanisms and multiple targets, are regarded to prevent AMR evolution. However, some reports raise warnings for the generation of AMR in bacteria exposed to antimicrobial nanomaterials, such as Ag,[18b] TiO2,[143] CuO,[144] ZnO,[145] CeO2,[146] Al2O3[147] and CNTs.[148] Based on these studies, we summarized the adaptation mechanisms of bacteria toward antimicrobial nanomaterials (Figure 3).

3.1 Microbial Adaptations to Nanomaterials
3.1.1 HGT
Bacterial cells may acquire resistance by nanomaterial-mediated HGT (Figure 3a). Although direct delivery of ARGs by nanomaterials into bacterial cells is questionable, nanomaterials provide a large immobilization surface for the interactions among bacteria, antibiotics, heavy metal ions and ARGs.[19b, 149] For instance, the plasmid transfer frequency displayed a 2-fold enhancement in the presence of multiwalled carbon nanotubes (MWCNTs), which could form aggregates to promote bacterial conjugation.[150] The relative abundance of tetracycline resistance genes in sludge and wastewater displayed a 1.04- to 1.54-fold increase after exposure to polystyrene micro/nanoplastics for 30 days.[151] In addition to carbonaceous nanomaterials, metallic nanomaterials, such as Ag,[152] CuO,[144] ZnO,[145] Al2O3,[147] Fe2O3,[153] TiO2,[143] and
CeO$_2$\cite{146} have been reported to exacerbate the horizontal conjugative transfer of plasmid-mediated multidrug resistance genes (e.g., RP4, RK2 and pCF10 plasmids). Pre-exposure to rare earth oxide NPs (La$_2$O$_3$, Nd$_2$O$_3$ and Gd$_2$O$_3$) in soil for 60 days could selectively enrich 40 ARGs, leading to increased resistance to tetracycline in soil microbes.\cite{154} Yu et al. suggested that the ARG propagation induced by CeO$_2$ could be attributed to the elevated production of extracellular polymeric substances as well as the increment of intracellular contact.\cite{146} Compared with TiO$_2$, SiO$_2$, and Fe$_2$O$_3$ NPs, Qiu et al. demonstrated that nano-Al elicited membrane damage, significantly promoted bacterial conjugation and facilitated the horizontal transfer of RP4.\cite{147}

### 3.1.2 Access Prevention of Released Ions

It is well known that bacteria can acquire or develop antibiotic resistance mechanisms by preventing their access to targets, involving reduced permeability and increased efflux systems.\cite{137} Likewise, bacterial cells can develop resistance to metallic nanomaterials by downregulating porins to reduce ion penetration\cite{20} or overexpressing efflux pump systems to transport ions\cite{18b} (Figure 3a). For instance, deficiency of OmpF/OmpC restricts the cytoplasmic internalization of Ag$^+$ in mutant \textit{E. coli} strains by impairing the expression of porins and the permeability of outer membranes. As a result, the mutant strain displayed > 8-fold higher MICs than wild-type \textit{E. coli}.\cite{20} Moreover, Stabryla et al. found that \textit{E. coli} K-12 MG1655 bacterial cells could develop heritable resistance to Ag NPs by mutating CusS,\cite{18b} which is a copper/silver ion sensor driving the expression of CusCBA efflux transporters to ameliorate toxic metal effects.\cite{155} Except for metal ions, there is no evidence showing that bacterial cells could prevent the access of nanomaterials to bacterial cells.
Figure 3. Bacterial adaptation mechanisms toward antimicrobial nanomaterials

Bacteria adapt to antimicrobial nanomaterials by a) bacterial adaptation, including i) HGT, such as ARG transformation, conjugation, and transduction; ii) access prevention of release ions, such as porin decrease and efflux pump overexpression; and iii) remodeling envelope adaptation, such as cell wall thickness, membrane rigidity increment and flagella/pili expression. b) Nanomaterial inactivation, including ionic precipitation, nanomaterial aggregation and degradation. Bacterial phenotypes are observable characteristics, including morphological, cultural, and biochemical features, e.g., shape, size and arrangement of bacterial cells, colony morphology, antigenic structure, pili, flagella, and metabolic properties.

3.1.3 Remodeling the Cell Envelope

To compromise the bactericidal effects of antimicrobial nanomaterials, bacterial cells may acquire resistance by remodeling cell envelope structures (membrane protein, lipid, LPS or PGN) (Figure 3a).[21,156] The cell envelope is often regarded as the first interface for nano-microbe interactions and functions as the primary barrier to avoid damage to bacteria from exogenous agents. Increasing the membrane rigidity by tuning the fatty
acid profile in membranes could reduce the punching effects of CNTs. To acquire such phenotypic changes, Gram-negative bacteria (E. coli and Ochrobactrum sp.) may elevate the proportion of saturated fatty acids, whereas Gram-positive bacteria may increase the ratio of branched-chain fatty acids vs. straight-chain fatty acids.\textsuperscript{[21]} The conformational change of unsaturated fatty acids from \textit{cis} to \textit{trans} results in more rigid membranes of \textit{Pseudomonas putida} cells after repetitive exposure to nanosized zero-valent iron.\textsuperscript{[157]} PGN and LPS layers could also be modified to alter bacterial membranes/walls in response to CeO\textsubscript{2} and Au treatments.\textsuperscript{[156, 158]} In addition to the membrane constituent change, bacterial cells may alter their morphologies, such as filamentation shape and cell wall thickness, to adapt to metallic nanomaterials, such as TiO\textsubscript{2} and nickel manganese cobalt oxide.\textsuperscript{[159]}

Compared to antibiotics, nanomaterials diffuse slowly and often form agglomerates. Long-term exposure to nanomaterials may result in a selection of high-motility bacteria to escape from danger. Thus, hypermobile \textit{E. coli} displays > 2-fold tolerance to Ag NPs compared with normal \textit{E. coli}.\textsuperscript{[18b]} After repetitive exposure of TiO\textsubscript{2} to \textit{E. coli}, more flagellar assembly and fimbria biosynthesis were detected in acquired cells to facilitate the production of longer flagella/pili to diminish bacterial susceptibility to oxidative stress\textsuperscript{[159b]} (Figure 3a).

### 3.2 Inactivation of Antimicrobial Nanomaterials by Metabolic Alterations

Inactivation of antimicrobial nanomaterials can be achieved by accelerating bacterial metabolic pathways to precipitate metal ions, degrade NPs, or induce agglomeration/crystallization (Figure 3b). The precipitation of released metal ions is an important adaptation mechanism in bacteria exposed to metallic nanomaterials.\textsuperscript{[160]} Naik \textit{et al.} identified two specific peptides that precipitate Ag\textsuperscript{+} and form face-centered cubic silver crystals.\textsuperscript{[161]} Meanwhile, to reduce nano-enabled oxidative damage, bacterial cells could exploit desired metabolic reactions to scavenge ROS by boosting antioxidant enzyme (superoxide dismutase and catalase) activity\textsuperscript{[153, 160a]} or upregulating the expression of the antioxidant riboflavin in \textit{Shewanella oneidensis} MR-
1 after exposure to nickel manganese cobalt oxide nanosheets with a stoichiometric Ni: Mn: Co of 1:1:1.\textsuperscript{[159a]} To reduce the disruption stress of antimicrobial nanomaterials, \textit{Trabusiella guamensis} released peroxidase to degrade MWCNTs by catalytically oxidizing the tube surface into C=O and C-OH groups;\textsuperscript{[162]} \textit{E. coli} cells secreted adhesion proteins, such as flagellin, to reduce the effective dosage of antimicrobial nanomaterials by inducing NP agglomeration.\textsuperscript{[18a]}

4 Specific Nano-microbe Interactions to Prevent AMR Evolution

Although resistance toward nanomaterials has been reported in some bacterial cells, a few antimicrobial nanomaterials have been explored to conquer resistant bacteria without triggering new resistance (Table 2), including metal-GO nanocomposites, Cu$_3$P, BN nanosheets, and five functionalized Au NPs. We discussed their interactions with bacteria to guide the design of antimicrobial nanomaterials for the prevention of AMR spread.

<table>
<thead>
<tr>
<th>Nano-materials</th>
<th>Strains</th>
<th>Mode of action</th>
<th>AMR evolution</th>
<th>Exposure time and dosage</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>La@GO</td>
<td>MDR \textit{E. coli}</td>
<td>EMTI mechanism</td>
<td>No resistance</td>
<td>30 days at 250 μg/mL</td>
<td>[18c]</td>
</tr>
<tr>
<td>GCN/Ag</td>
<td>\textit{E. coli}</td>
<td>membrane disruption and protein dysfunction</td>
<td>MIC increases from 3.4 to 7 μg/mL</td>
<td>60 days at sub-MIC</td>
<td>[26a]</td>
</tr>
<tr>
<td>BN</td>
<td>MDR \textit{E. coli}</td>
<td>impairment of Z-ring constriction</td>
<td>No resistance</td>
<td>30 days at 125 μg/mL</td>
<td>[16b]</td>
</tr>
<tr>
<td>Cu$_3$P</td>
<td>\textit{E. coli}</td>
<td>membrane damage, ROS generation, GSH depletion</td>
<td>No resistance</td>
<td>3 days at 0.8 μg/mL</td>
<td>[167]</td>
</tr>
<tr>
<td>QA-Au</td>
<td>\textit{S. aureus}</td>
<td>membrane disruption</td>
<td>No resistance</td>
<td>30 days at 2.5 μg/mL</td>
<td>[166a]</td>
</tr>
<tr>
<td>DAPT-Au</td>
<td>\textit{E. coli}</td>
<td>membrane disruption, dysfunction of DNA</td>
<td>MIC increases from 6 to 8 μg/mL</td>
<td>21 passages at 4 μg/mL and then 30 passages at 6 μg/mL</td>
<td>[32]</td>
</tr>
<tr>
<td>BSA-Au</td>
<td>\textit{E. coli}</td>
<td>oxidase-like activity for ROS production</td>
<td>Slow resistance</td>
<td>32 days at 1-4 μg/mL</td>
<td>[142]</td>
</tr>
<tr>
<td>BSA-DAPT-Au</td>
<td>\textit{E. coli} and \textit{S. aureus}</td>
<td>membrane disruption</td>
<td>No resistance</td>
<td>32 days at 0.25-0.5 μg/mL in \textit{E. coli} and at 4-8 μg/mL in \textit{S. aureus}</td>
<td>[165a]</td>
</tr>
<tr>
<td>Decane-AuNPs</td>
<td>\textit{E. coli}</td>
<td>membrane disruption</td>
<td>No resistance</td>
<td>20 passages at 10.56 μg/mL (16 h/passage)</td>
<td>[128]</td>
</tr>
</tbody>
</table>

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Note: the abbreviations in the table are illustrated as follows: EMTI (extracellular multitarget invasion); GCN (cyanographene); QA (quaternary ammonium); DAPT (4,6-diamino-2-pyrimidinethiol).

4.1 Extracellular Attack of Conserved Envelope Constituents

Bacterial cells evolve sophisticated mechanisms to invalidate intracellular bactericides (e.g., β-lactams or aminoglycosides) by mutations coding for enzymes. However, the adaptation capability of bacteria to a new antibiotic, teixobactin, is very limited,\textsuperscript{[29]} possibly due to its highly conserved binding sites in lipid II (precursors of PGN). Inspired by this work, antimicrobial nanomaterials have been designed to interact with the conserved sites of constituents in the cell envelope. For instance, Zheng et al. synthesized a lanthanum hydroxide and graphene oxide nanocomposite (La@GO) by growing La(OH)\textsubscript{3} crystals in GO nanosheets to simultaneously invade the bacterial membrane/cell wall by stripping phosphate groups from membrane lipids to precipitate with La\textsuperscript{3+}, oxidizing unsaturated fatty acids and hydrolyzing PGN on GO surfaces (Figure 4a-c).\textsuperscript{[18c]} Strikingly, after 30 days of exposure of La@GO to MDR E. coli, neither nano-resistance (Figure 4d) nor cross-resistance (Figure 4e) was detected. In comparison, norfloxacin antibiotic and Ag NPs displayed 64- and 16-fold increments of MICs, respectively (Figure 4d).\textsuperscript{[18c]} Graphene materials covalently conjugated with nanosilver (GCN/Ag) displayed a similar mechanism to La@GOs and significantly diminished silver resistance with only a marginal increase in MIC from 3.4 to 7 mg/L after 60 passages.\textsuperscript{[26a]} Notably, although these nanocomposites show strong killing efficiency without triggering AMR evolution, there are still challenges in clinical use because their targets are often expressed in both prokaryotes and eukaryotes. A recent finding by Pan et al. provides an imaginative solution to overcome that limitation by exploring antibiotic-like nanomaterials, \textit{i.e.}, nano-antibiotics. Interestingly, BN nanosheets were identified to display AMR-independent killing activity in bacteria and extraordinary biocompatibility in mammalian cells.\textsuperscript{[16b]} This material was capable of binding to the conserved sites of surface proteins (\textit{e.g.}, FtsP, EnvC, TolB), which are responsible for Z-ring constriction/degradation in bacterial cell division. This interaction leads to cycle arrest and prevents colonies of resistant \textit{P. aeruginosa} from
infecting mouse lungs. Long-term exposure of BN nanosheets to MDR *E. coli* did not trigger secondary detectable resistance.[16b] Overall, this mode of action opens new avenues to explore antimicrobial nanotechnologies for the prevention of AMR spread. Instead of targeting phospholipids, promising nano-antibiotic candidates should be designed to attack the conserved sites of biomarkers on the bacterial surface, such as Lipid II,[29] PGN, LPS, BamA,[163] MraY,[164] etc.

Figure 4. Engineering La@GO nanocomposites for preventing AMR evolution

**a**) Super-resolution confocal images. The PGN layer of MDR *E. coli* was labeled by a fluorescent D-amino acid *in situ* and observed by super-resolution microscopy after treatment with La@GO. **b**) nano-CT images of MDR *E. coli* treated with La@GO. **c**) Illustration of the EMTI mechanism. **d**) AMR evolutonal trajectory in MDR *E. coli*;
AMR evolution test was employed by exposure of La@GO, Ag NPs, and norfloxacin to MDR *E. coli* for 30 days under sub-MIC. e) Assessment of cross-resistance in acquired *E. coli* after 30 days of exposure. Reprinted with permission from ref [18c]. Copyright {2021} American Chemical Society.

### 4.2 Parallel Interactions with Multiple Targets

Although resistant bacterial cells can rapidly mutate to avoid attacking a specific target, simultaneous mutations of multiple independent biomolecules are rarely reported. This offers new insights into overcoming AMR evolution by exploiting the heterogenic bactericidal actions of antibiotic cocktails. Coincidentally, heterogenicity is a distinct feature of nanomaterials compared to chemical compounds. It allows parallel and multivalent interactions of nanomaterials with multiple independent targets by different forces. Jiang and coworkers demonstrated this by developing a battery of functionalized Au NPs with 4,6-diamino-2-pyrimidinethiol (DAPT),[^32] [158] [165] BSA[^142] or quaternary ammonium (QA)[^166] (Figure 5a-c). In contrast to the rapid acquisition of resistance to ampicillin, the oxidase-like activity of BSA-Au and QA-Au boosted diverse oxidative species, such as $\cdot$O$_2$, $\cdot$OH, and $O_2$$\cdot$-, to react with different intracellular targets, contributing to the robust anti-superbug (Figure 5d) and anti-biofilm abilities (Figure 5e) without inducing AMR evolution over 30 days of exposure.[^142] [166a] Although antioxidative systems may have evolved in bacteria to neutralize the accumulated ROS, the durable catalytic effects as well as parallel interactions with multiple independent targets decisively delay the onset of AMR evolution. Consistently, Cu$_3$P NPs with oxidase- and peroxidase-like activities could induce > 99.9% killing efficiency against *E. coli* and MRSA at a low concentration by GSH depletion and lipid peroxidation without triggering resistance[^167] In addition to these oxidase/peroxidase-like nanozymes, other enzymatic activities have been reported to catalyze specific biomolecules, such as CeO$_2$ NPs with dephosphorylation activity,[^72] SnSe nanosheets with dehydrogenase-like activity,[^70] and metal-doped graphene nanosheets with NAD(P)H oxidase activity.[^69] [168]
**Figure 5. Engineering capped Au to prevent AMR evolution**

**a)** Evolutional trajectory of *E. coli* in the presence of BSA-Au and ampicillin. Reprinted with permission from ref[142]. Copyright {2022} American Chemical Society.

**b)** MIC changes of DAPT-Au against *E. coli*, *P. aeruginosa* and *K. pneumoniae*; Reprinted with permission from ref[158]. Copyright {2021} American Chemical Society.

**c)** Evolutional trajectory of *S. aureus* in the presence of QA-Au and oxacillin; the AMR evolution test was performed by exposure of capped Au NPs to bacterial cells for serial passages under sub-MIC.

**d)** TEM images of *Staphylococcus aureus* treated with QA-Au. Bacterial cells were exposed to QA-Au at different concentrations and observed by TEM. Reprinted with permission from ref[166a]. Copyright {2018} Wiley.

**e)** Biofilm images of *P. aeruginosa* and MRSA with BSA-Au treatment. After incubation of BSA-Au...
Au with the bacterial cells for 96 h, the biofilm was stained by SYTO 9 and observed by confocal microscopy with Z-Track software. Scale bar: 100 μm. Reprinted with permission from ref[142]. Copyright {2022} American Chemical Society.

4.3 Nano-enabled Physical Stress

Before the discovery of antibiotics, pathogens were disinfected by extreme physical circumstances (e.g., ultraviolet light, high temperatures, and vacuum) as alternatives to exposure to reactive species without major resistance development. Intriguingly, such physical stimuli can be created by designed nanomaterials to control photodynamic, photothermal, sonodynamic and magnetic hyperthermia activities. For instance, a positively charged subphthalocyanine nanosphere (SubPc) could serve as a photosensitizer to boost local singlet oxygen under 600 nm radiation to kill bacteria.[169] Considerable nanomaterials, including metal NPs (e.g., TiO₂,[170] AuPt[105]), carbon nanomaterials (e.g., CNTs, g-C₃N₄, GQDs[34] and heterostructure nanocomposites (e.g., ZnO/CQDs/g-C₃N₄, MnO₂/g-C₃N₄[26b]), have been found to show excellent photocatalytic antibacterial effects. Moreover, photothermal therapies by CuS,[171] Au,[172] Fe₃O₄@MoS₂,[173] CNTs,[174] and graphene derivates[175] can efficiently elevate the local temperature under photon irradiation. Yu et al. constructed a supramolecular nanocomposite made of graphene nanoribbons and a cationic porphyrin displaying photodynamic and photothermal activities.[176] Taking advantage of sonodynamic activity, cavitation, oxidative and thermal effects[177] could be exploited to combat MDR bacteria by functionalized nanomaterials, such as maltohexaose-modified liposomes encapsulating purpurin 18[178] and Ti₂C(OH)₂ decorated with TiO₂ nanofibers.[179] In addition, diverse magnetic nanomaterials (e.g., Fe, Ni, Co, Fe₃O₄ NPs) have been synthesized with well-controlled magnetic hyperthermia activity for disinfection.[180] These nanomaterials act as an energy transducer to convert biocompatible radiation into bactericidal signals, which can be exploited for precise killing of pathogens by controllable spatiotemporal-responsive activity. However, more investigations should be focused on their impacts on AMR evolution.
5 Principles to Design Antimicrobial Nanomaterials for Prevention of AMR Evolution

The varying claims for the impact of nanomaterials on AMR evolution can be attributed to the diversity of antimicrobial nanomaterials, resulting in distinct nano-microbe interactions and antimicrobial mechanisms. This requires specific design and cautious application of antimicrobial nanomaterials. According to the acquired knowledge of nano-microbe interactions, antimicrobial pathways, and SARs, we propose four design principles to fabricate antimicrobial nanomaterials for the prevention of AMR evolution, including engineering nanocomposites to diversify nano-microbe interactions, stimuli-responsive functionalization for controllable bactericidal effects, grafting of targeting ligands for precise bacterial killing, and doping/shielding to avoid ion release (Figure 6).

5.1 Engineering Nanocomposites to Diversify Nano-microbe Interactions

As stated before, nanomaterials with parallel interactions and multiple targets display suppressed risks of inducing AMR evolution. To acquire such antimicrobial nanomaterials, multiple functionalization is preferred to diversify the interactions between nanomaterials and bacterial cells, such as structural destruction of biomolecules in bacteria, catalysis of bacterial metabolites to generate toxic species and noncovalent binding with bacterial membrane constituents. Compared to separate NPs, the components of nanocomposites often display synergistic bactericidal effects, especially when they have different interactions or targets. For instance, rare earth NPs are known to react with phosphate groups on phospholipids of membranes,[181] while GOs are able to induce lipid peroxidation and PGN hydrolysis. A rational decoration of La(OH)₃ on GO surfaces could induce an over 4-fold increase in bacterial killing and did not elicit any detectable gene mutations after 30 days of exposure.[18c] In addition, multiple enzymatic nanomaterials could be prepared by loading Ce on MOF to display the activities of hydrolase, peroxidase and oxidase. This functional material can degrade extracellular substances (extracellular DNA) and generate oxidative species to kill bacteria, which is extremely important for the prevention of biofilm formation.[141]
Constructing heterostructures between carbon nanomaterials (e.g., GO, g-C₃N₄, QDs) and metal oxide NPs might be another promising strategy to diversify and increase the photocatalytic properties of nanocomposites, such as sulfonated GO-ZnO-Ag, ZnO/CQDs/g-C₃N₄, and MnO₂/g-C₃N₄.[26b] These nanocomposites displayed enhanced light absorption and efficient charge separation due to their hierarchical structure. This is a generic design principle that could be applied to many nanomaterials, including carbonaceous and metallic NPs. However, the components of designed nanocomposites should be cautiously selected to display distinct interactions with bacteria.

5.2 Stimuli-responsive Functionalization for Controllable Bactericidal Effects

The widespread AMR may also be attributed to the high levels of residual antibiotics in the environment. Learning from this lesson, antimicrobial nanomaterials should be designed with stimuli-responsive moieties to precisely interact with target bacteria and reduce their residual amounts in real scenarios. To minimize the contamination of Au@Ag nanocomposites in aquatic environments, an Fe₃O₄ core was designed for the efficient recycling of NPs in water treatment by an external magnetic field.[182] Light-control activity is another promising strategy to eliminate the residual bactericidal effects of antimicrobial nanomaterials by grafting photoresponsive moieties, such as the conjugation of photoconverted azobenzenes with biocidal β-cyclodextrin derivatives[183] and the incorporation of photosensitized porphyrin into MOFs.[184] According to the differences in stimuli, responsive moieties can be divided into two types. One is controlled by external radiation, such as magnetic fields and photons, and the other can respond to the intrinsic biochemical features of the microbial community, such as pH-responsive[185] and enzyme-responsive nanomaterials.[186] These nanomaterials could be used for controllable killing of bacterial cells in biofilms because the microbial community often displays a specific microenvironment, such as low oxygen and pH and sufficient hydrolytic enzymes. This design strategy could be applied to almost all nanomaterials.
Figure 6. Four proposed principles for designing antimicrobial nanomaterials

The design of antimicrobial nanomaterials without triggering AMR evolution should consider i) engineering nanocomposites to diversify nano-microbe interactions, such as structural destruction of biomolecules, catalysis of bacterial metabolites and noncovalent binding with bacterial membrane constituents; ii) stimuli-responsive functionalization (e.g., pH, magnet, light and enzyme) for controllable bactericidal effects; iii) grafting of targeting ligands (e.g., charged ligands, aminos, peptides, polysaccharides, and proteins) for precise bacterial killing; and iv) doping/shielding modification, including surface coating, core-shell construction and doping, to avoid metal ion release.

5.3 Grafting of Targeting Ligands for Precise Bacterial Killing

Engineering narrow-spectrum antimicrobial nanomaterials is a preferable choice for specifically killing pathogenic bacteria, selectively protecting beneficial bacteria and preventing broad-spectrum resistance. This could be performed by grafting desired
ligands (e.g., combinatorial charged groups, peptides, polysaccharides, and proteins) onto the particle surface to achieve Gram- or species-selectivity. Gram-selective antibacterial nanomaterials are often constructed to recognize the specific constituents (e.g., LPS and lipoteichoic acid)\(^{[188]}\) and structural features (surface charge\(^{[125]}\) and roughness) of cell walls. Customized nanomaterials with different antibacterial properties could be prepared by regulating the molar ratio or density of surface ligands.\(^{[187-188]}\) In addition, while AMR biomarkers have been extensively identified in bacteria, it is possible to graft antimicrobial nanomaterials with targeting antibodies to improve the killing efficiency against AMR cells and selectivity between eukaryotes and bacteria.\(^{[189]}\) Moreover, narrow-spectrum peptides could be engineered into antimicrobial nanomaterials to recognize and bind with specific bacteria, achieving selective killing of pathogens.\(^{[52, 190]}\) The targeting ligands could either be covalently conjugated or noncovalently assembled with nanomaterials. This design principle could be applied to inorganic (Ag, CuO, GO, etc.) and organic (liposomes, micelles, etc.) nanomaterials.

## 5.4 Doping/Shielding to Avoid Ion Release

While most metallic nanomaterials are often present in a heterogenic state consisting of metal ions and particulates in biocontexts, the released ions play an important role in the killing effect of nanomaterials. However, metal ions generally exhibit indiscriminate toxicity in eukaryotes and trigger defense mechanisms in bacterial cells to compromise their bactericidal effects. As discussed before, bacterial cells could quickly evolve resistance in the presence of metal ions, such as silver, copper, zinc and cadmium ions. Prevention of ion release from metallic nanomaterials may reduce the risk of AMR evolution. Since ion release results from surface defects or redox reactions with biochemical molecules, the stability of nanomaterials in biological contexts could be enhanced to avoid the leakage of metal ions by doping\(^{[191]}\) or shielding (surface coating,\(^{[192]}\) implanting\(^{[26a, 193]}\) or constructing core-shell structures\(^{[194]}\)). Rational selection of dopants could alter the host lattice or band gap of nanomaterials to diminish their reactions with surrounding media, such as doping iron\(^{[191]}\) with ZnO nanomaterials.
Chelators (e.g., ethylenediamine tetra(methylene phosphonic acid)\textsuperscript{[192b]}, polymers (e.g., polyvinyl pyrrolidone,\textsuperscript{[195]} polyethylene glycol (PEG),\textsuperscript{[196]} PEI\textsuperscript{[197]}) and amphipathic compounds\textsuperscript{[198]} have been extensively exploited as coating materials to increase the stability of NPs and reduce ion shedding. Construction of the core-shell structure is regarded as the most efficient approach to reduce ion release in many metallic NPs, such as Ag@SiO\textsubscript{2} colloids\textsuperscript{[199]} and Fe\textsubscript{3}O\textsubscript{4}@Alyssum homolocarpum seed gum@Ag nanocomposites.\textsuperscript{[194a]} However, this approach may be inapplicable to antimicrobial nanomaterials that require a direct interaction/association with bacteria, as the shell layer is often composed of inert materials.

6 Summary and Outlook

While the exploration of antimicrobial nanotechnology is still burgeoning, diverse antimicrobial nanomaterials are increasingly applied in biomedicine, food package, water treatment, etc. In addition to the differing impacts on AMR evolution, antimicrobial nanomaterials face other challenges that restrict their applications in more areas, such as poor selectivity, insufficient antimicrobial efficiency, side effects, and low colloidal stability.

- **Poor Selectivity.** Antibiotics are often derived from the natural metabolites of bacterial cells that are exquisitely refined to selectively target specific bacterial strains without harming the infected host. In contrast, most nanomaterials are artificially synthesized in endothermal reactions, resulting in a reduction in entropy and an increase in enthalpy. Therefore, nanomaterials often have high surface energy to bind with diverse biochemical molecules, leading to adverse effects. For instance, Ag NPs exhibit significant cytotoxicity due to nonspecific adsorption and accumulation in macrophages.\textsuperscript{[200]} In addition, the poor selectivity of nanomaterials may result from their heterogeneous constituents. Although substantial progress has been made for controlled synthesis in nanoscience, it is difficult to acquire standardized nanomaterials with specific physicochemical properties. Almost any type of synthesized nanomaterial may contain some heterogeneous particulates with different sizes, surface chemistry, or...
morphology, leading to different biological fates and responses. Moreover, even though some antimicrobial nanomaterials are claimed to interact with specific targets, most of them are conserved biomolecules such as membrane lipids, DNA, and GSH. However, these biomolecules are commonly expressed in biological systems, leading to similar toxic events in eukaryotes and prokaryotes. In summary, the poor selectivity of nanomaterials could be attributed to nonspecific binding on their surface, heterogeneous biological effects, and conserved interaction targets.

- **Insufficient Antimicrobial Efficiency.** The nano-microbe interactions often involve one or a few critical biochemical reactions that dictate the antimicrobial activity of nanomaterials. The reaction efficiency is determined by thermodynamics and kinetics, both playing critical roles in antimicrobial activity. Although some nanomaterials are more active than their molecular counterpart, they may have low reaction efficiency with their targets in bacterial cells because collision theory is regarded to dictate the reaction rate. Antimicrobial nanomaterials have lower diffusion rate and smaller number concentrations, resulting in a lower collision probability with bacteria and insufficient killing efficiency.

- **Biosafety Concerns.** Nanomaterials display distinct adsorption, distribution, metabolism and excretion behaviors in animals. On the one hand, more efforts are required to standardize the biosafety assessment of antimicrobial nanomaterials. On the other hand, the poor selectivity of antimicrobial nanomaterials may elicit inevitable toxicity in animals.

- **Low Colloidal Stability.** Antimicrobial nanomaterials are often present in the colloidal state, which is very sensitive to changes in temperature, pH, ionic strength, etc. Disruption of colloidal stability leads to nanomaterial aggregation and may affect their antimicrobial activity.

This review overviews a few knowledge gaps in the differing impacts of antimicrobial nanomaterials on AMR evolution. We highlight the nano-microbe interactions to understand the underlying mechanisms of prevention or aggravation of AMR evolution. It provides distinct insights into the designs and applications of antimicrobial
nanomaterials. Overall, considerable efforts should be made to rationally construct antimicrobial nanomaterials to fight against superbugs without AMR evolution. Herein, we propose perspectives on the development of antimicrobial nanotechnology for AMR prevention.

**Identification of Nano-adjuvant to Eradicate AMR Bacteria.** Combining antibiotics with potentiating adjuvants is one solution that can enhance the potency of antibiotics. Nano-adjuvants are nanomaterials that are able to potentiate ineffective/impermeable antibiotics.\(^{[201]}\) Therefore, nano-adjuvants are often used in conjunction with antibiotics by adsorption or covalent conjugation for antimicrobial uses. Recently, only a few nanomaterials, *e.g.*, silver nanoclusters, selenium nanowires modified with Ag, and gold nanoclusters, have been found to display synergistic antibacterial effects with ampicillin, kanamycin, norfloxacin and ofloxacin.\(^{[202]}\) These formulas were applied to eradicate resistant bacteria or persisters by blockage of bacterial respiratory metabolism, disruption of the proton gradient or membrane hyperpolarization.\(^{[202b, 202c, 203]}\) Compared to other adjuvants, nanomaterials have three advantages: i) a large compatible surface to load antibiotics; ii) diverse nanobiological effects; and iii) prolonged and controllable release of antibiotics. However, more efforts are required to overcome the limitations of nano-adjuvants, including i) the nano-adjuvants cannot fully reverse or retard AMR evolution and ii) the nano-adjuvants may elicit cytotoxicity.

**Catalytic Quenching of Bacterial Communications.** Since traditional bactericidal agents directly interacting with microbes may inevitably lead to AMR evolution, engineering contactless antimicrobial nanomaterials may retard these issues. Nanocatalysts with enzyme-like activities under physiological conditions, *i.e.*, nanozymes, are promising candidates. Given that bacterial cells secrete specific communication signals in the surrounding environment to establish a local community (*e.g.*, biofilm), nanozymes toward these biomarkers, such as bacterial quorum sensing (QS) signals,\(^{[204]}\) can be exploited to disrupt cell communications and slow their growth. While N-acylated-L-homoserine lactone (AHL) and autoinducer peptide (AIP) are two
representative QS signals, the bacterial communication systems supposedly could be blocked by hydrolase- or peptidase-like nanozymes to prevent microbial proliferation.

**Design of Antibiotic-like Nanomaterials for Selective Killing.** Pan et al. used an interesting term, “nano-antibiotics” to describe these antibiotic-like nanomaterials.\(^{[16b]}\) Notably, this term may also represent antibiotics fabricated in the form of nanomaterials. However, “nano-antibiotics” used in this review merely refers to antibiotic-like nanomaterials, while antibiotic-nanomaterial formulas are not included. Some antimicrobial nanomaterials display comparable killing efficiency to antibiotics, but their selectivity toward eukaryotes and prokaryotes is still poor. To conquer this challenge, antimicrobial nanomaterials must be refined with bioinert surfaces to reduce nonspecific binding with biomolecules, homogeneous particulates to avoid undesired effects, and the recognition capability to interact with specific biomarkers in certain bacterial strains (Figure 7). A few successful attempts have been made in chemotherapy to reduce nonspecific binding on drug carriers and prolong the blood circulation of cargo molecules. These engineering approaches, such as PEGylation, human albumin conjugation, and phospholipid coating, could be adopted to functionalize antimicrobial nanomaterials. While it is premature to acquire homogeneous nanomaterials by controlled synthesis, some advanced purification techniques, such as free-flow electrophoresis\(^{[205]}\) isoelectric focusing electrophoresis\(^{[206]}\) and size exclusion chromatography,\(^{[207]}\) could be exploited to acquire uniform nanomaterials. We recommend two types of functionalization on these purified nanomaterials to prepare nano-antibiotics for targeting bacteria. First, inspired by the infection manner of bacteriophages, antibodies, aptamers and molecular ligands targeting surface biomarkers could be grafted onto nanomaterials to recognize specific bacterial strains. Second, the selectivity of antimicrobial nanomaterials could be achieved by molecular imprinting, by which target-shaped cavities could be created on the nanomaterial surface. These imprinted nanomaterials could recognize specific biomarkers by steric hindrance and noncovalent interactions, including hydrogen bonds and ionic, hydrophobic, and Van der Waals forces, which is similar to antibody-antigen...
interactions. Currently, although diverse imprinted nanomaterials have been prepared, few attempts have been made to design antimicrobial nanomaterials by this technique. The rational design of nano-antibiotics may be an attractive strategy to conquer the AMR crisis.

Figure 7. Construction steps of antibiotic-like nanomaterials
The construction of antibiotic-like nanomaterials includes three steps. First, the active surface of nanomaterials is passivated by coating of bioinert ligands. Second, the coated materials are purified by electrophoresis or chromatography separation according to their differences in size and surface chemistry. Third, the purified nanomaterials are functionalized to conjugate targeting ligands or create recognition sites by molecular imprinting.

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This review focuses on the impacts of antimicrobial nanomaterials on the evolution of antimicrobial resistance (AMR). The relationships among nanomaterial structures, bactericidal effects and AMR evolution are thoroughly discussed to summarize the design principles of desired antimicrobial nanomaterials for the prevention of AMR spread.

**Twitter:** @ruibin_lab
Maomao Xie received her B.S. degree from Soochow University in 2020. She is currently a M.S. student in Soochow University. Her research interests focus on nanomaterials and antimicrobial resistance.

Meng Gao received Ph.D. degree in 2017 from Dalian Institute of Chemical Physics, Chinese Academy of Sciences. She is currently an associate professor in Soochow University. Her research interests focus on: i) developing new nanozymes for biological applications; ii) discovering new antimicrobial methods for antibiotic resistant bacteria.

Yang Yun received Ph.D. degree in 2011 from Shanxi University. She is a professor in College of Environment and Resources, Shanxi University. Her research interests focus on: i) toxicology of micro- and nanoplastics; ii) evolution of antimicrobial resistance in bacterial communities.
Huizhen Zheng received Ph.D. degree in 2017 from Dalian Institute of Chemical Physics, Chinese Academy of Sciences. She is an associate professor in Soochow University. Her research interests include: i) structure-activity relationships of nanomaterials; ii) development of nanomaterials for combating antimicrobial resistance evolution.

Ruibin Li is a professor in State Key Laboratory of Radiation Medicine and Protection, School of Radiation Medicine and Protection, Soochow University. His research interests focus on: i) understanding the nano-bio interactions; ii) nanotoxicology; iii) development of antimicrobial nanomaterials.