Published in final edited form as:

J Mater Chem B. 2020 October 28; 8(41): 9466–9480. doi:10.1039/d0tb00549e.

Development of coinage metal nanoclusters as antimicrobials to combat bacterial infections

Dan Li^{b, #}, Beena Kumari^{c, #}, Jessa Marie Makabenta^a, Bailong Tao^d, Kun Qian^b, Xifan Mei^b, Vincent M. Rotello^a

^aDepartment of Chemistry, University of Massachusetts Amherst, 710 North Pleasant Street, Amherst, Massachusetts 01003, USA.

^bDepartment of Basic Science, Jinzhou Medical University, 40 Songpo Road, Jinzhou 121001, China

^cDepartment of Chemistry, Indian Institute of Technology Gandhinagar, India

^dCollege of Bioengineering, Chongqing University, Chongqing 400044, China

Abstract

Infections from antibiotic-resistant bacteria have caused huge economic loss and numerous deaths over the past decades. Researchers are exploring multiple strategies to combat these bacterial infections. Metal nanomaterials have been explored as therapeutics against these infections owing to their relatively low toxicity, broad-spectrum activity, and low bacterial resistance development. Some coinage metal nanoclusters, such as gold, silver, and copper nanoclusters, can be readily synthesized. These nanoclusters can feature multiple useful properties, including ultra-small size, high catalytic activity, unique photoluminescent properties, and photothermal effect. Coinage metal nanoclusters have been investigated as antimicrobials, but more research is required to tap their full potential. In this review, we discuss multiple advantages and the prospect of using gold/silver/copper nanoclusters as antimicrobials.

1 Introduction

Bacterial infections cause more than a million cases of illnesses and ~20 thousand deaths annually in the United States, impacting the economy by \$50–70 billion per year.^{1, 2} Antibiotics such as streptomycin, penicillin, tetracycline, and vancomycin are widely used to combat bacterial infections.^{3, 4} These traditional antibiotics can have limitations such as diminished stability in the body and other side effects.⁵ Additionally, drug resistance has been developed over time by the bacteria rendering antibiotics ineffective. Multiple strategies are used to overcome antibiotic resistance, such as the development of new drugs,

rotello@chem.umass.edu.

[#]Both authors contribute equally to this work.

Publisher's Disclaimer: Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this Accepted Manuscript with the edited and formatted Advance Article as soon as it is available.

Conflicts of interest

There are no conflicts to declare.

high-dose administration of antibiotic agents, and the combination of multiple antibiotics.⁶ However, these efforts are unable to keep up with the fast spread and development of resistance, and toxicity has been associated with ‘last resort’ antibiotics and high dose treatments.⁷ The illness and deaths due to antibiotic-resistant infections is an urgent issue,^{8–10} with the generation of multidrug-resistant (MDR) bacteria seriously increasing morbidity and mortality.^{11, 12}

Metal nanoparticles (NPs) show excellent antibacterial activities while overcoming drug-resistance barriers and other limitations associated with standard antibiotic strategies.^{13–23} However, most of these NPs-based antimicrobials have sizes ranging from 5 – 100 nm.^{24, 25} The relatively large NPs increase bioaccumulation.²⁶ For instance, silver nanoparticles (AgNPs) are one of the most effective nanoweapons to combat bacterial infections, but most AgNPs are difficult to be efficiently cleared from the organs.²⁷ In this aspect, much smaller nanomaterials with higher clearing efficiency may be favorable.^{28–30}

2 Coinage metal nanoclusters (NCs)

Metal nanoclusters (NCs) feature a metal core protected by ligands, which have molecule-like properties that bridge the gap between the traditional NPs and molecular systems.^{31–40} The metal core is comprised of a single element or combinations of different elements with a small number of atoms.^{41–44} Many ultra-small nanomaterials can be readily oxidized, causing instability.^{45–48} Coinage metals, such as gold (Au), silver (Ag), and copper (Cu), are resistant to oxidation. Stable NCs can be readily and cost-effectively obtained from these coinage metal precursors.⁴⁹ Currently, most NCs-based biomedicines are based on Au, Ag, and Cu, which are all coinage metal NCs. This is because these NCs have low toxicities, unique fluorescent properties, and useful catalytic activities. For instance, we obtained water-soluble copper NCs (CuNCs) (< 2 nm) in an aqueous medium using a one-pot method (Figure 1).⁵⁰ The resulting solution shows high catalytic activity. Compared to the non-fluorescent CuNPs,⁵¹ the CuNCs have tunable fluorescence, with wavelength relating to the number of copper from the metal core and the surface properties (Figure 1a, a1).

Coinage metal NCs can have advantageous properties over corresponding NPs for biomedical applications. For instance, the smaller gold NCs (AuNCs) can be cleared from the body much more easily than those larger gold NPs (AuNPs).^{52–54} Smaller NCs also metabolize more efficiently in the body than the larger NPs.^{55, 56} Based on these properties, AuNCs have been investigated for *in vivo* disease therapy such as Parkinson’s disease, and cancer using mice models^{57, 58}

Coinage metal NCs can efficiently interact with the bacterial cells via direct/indirect conjugation, ligand binding, and carrier-dependent cell internalization.^{59, 60} This interaction facilitates the design of bacterial probes with great potential for the diagnosis of bacterial infections both *in vitro* and *in vivo* using NCs.⁶¹ It is worth noting that this interaction can also kill bacteria.^{62–64} For instance, some AuNCs, AgNCs, and CuNCs have shown excellent antibacterial activity.⁶⁵

We have reviewed here the use of these three coinage metal NCs for the eradication of bacteria. Multiple mechanisms that play critical roles in designing nanocluster-based antimicrobials will be discussed. The promising aspects that make them useful for antimicrobials, including multiple advantageous aspects relative to NPs will also be elaborated (Figure 2).

3 Antimicrobial Design

3.1 Surface ligands

The surface chemistry of NPs influence NP-bacterial interactions, impacting their toxicity.⁶⁸ Quaternary ammonium (QA) compounds have been used to impart antibacterial activity to nanomaterials.^{69, 70} For instance, Huda *et al.* synthesized AgNPs protected by dodecylamine. The resulting AgNPs were then functionalized on AuNPs by a complicated ligand exchange process to produce a coating with long-term antibacterial activity.⁷¹ On the other hand, QA-functionalized glutathione (GSH) protected AuNCs (GSH-AuNCs) could be fabricated by a one-pot method by simply mixing QA, GSH, and HAuCl₄ (Figure 3).⁷² The as-obtained mixtures of QA-AuNCs were directly used for *in vitro* and *in vivo* antibacterial applications, showing high antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA). AgNCs can also be obtained by a one-pot method and used directly as antimicrobials. For instance, both dihydrolipoic acid (DHLLA) and GSH protected AgNCs were synthesized in aqueous solutions then used for the eradication of *Escherichia coli* (Table 1). AgNCs show comparable antibacterial activity to the AgNPs protected by the same ligand, but the procedures for fabricating AgNPs are generally more complicated. These readily-obtained NCs facilitate a wide range of life science applications.

3.2 Surface Charges

Bacteria generally have negative surface charges and interact with positively charged NPs by electrostatic interactions.⁷⁷ Positively charged surfaces endow NCs with antibacterial effects. For Instance, Ag⁺-rich AgNCs interacted with the bacterial cell walls more efficiently than Ag⁰-rich AgNCs, which consequently induce the release of more Ag⁺, enhancing reactive oxygen species (ROS) levels.^{78, 79} Therefore, Ag⁺-rich AgNCs show high antimicrobial activity towards both Gram-positive (*B. subtilis* and *S. aureus*) and Gram-negative (*P. aeruginosa* and *E. coli*) bacteria.⁸⁰ However, the increased Ag⁺ also raises issues of cytotoxicity, as Ag⁺ is toxic to mammalian cells, resulting in lysosomal damages and dysfunction of cellular targets.^{81, 82} To get around this limitation, the surface charges and the antibacterial activity have been modified with the functionalization of cationic polymers on AgNCs. We found that AgNCs displayed more excellent antibacterial effects against 12 multidrug-resistant uropathogenic strains of bacteria after the functionalization of cationic polyethylenimine (PEI).⁸³ In another work, PEI-conjugated AgNCs also showed enhanced antibacterial activity against *E. coli* over AgNCs.⁸⁴ Unlike Ag⁺, the cationic charges provided by the polymers exhibit high antibacterial efficiency while demonstrating low toxicity towards mammalian cells. These findings reveal that the more positive the charge, the better will be the antibacterial effects.⁶⁸ However, Xie's group suggested a stark contrast to this paradigm, showing that more negatively charged AuNCs were found to exhibit better antibacterial effects due to the elevated ROS level.⁸⁵ Figure 4 compared the antibacterial

activity of the AuNCs with different charges. The 6-mercaptophexanoic acid (MHA) protected AuNCs (Au₂₅MHA₁₈) with the most negative zeta potential, demonstrated the highest antibacterial activity, based on the highest death ratio of bacteria.

Overall, NCs can be easily functionalized with different ligands to enhance antimicrobial effects. Both positively and negatively charged NCs can show high antibacterial activities by careful NCs design.

4 Antibacterial mechanisms

Antibacterial NPs can access multiple bacteriostatic and bactericidal mechanisms such as cell wall disruption, internalization and interference with the intracellular process. These mechanisms are also employed by antimicrobial NCs.^{22, 23, 67, 81, 86} With NCs, the internalized metal ions can damage intracellular components and disrupt cellular functions. Additionally, the increase in ROS of cells induced by the NCs can cause bacterial cell damage. The mechanisms for the eradication of bacteria by typical NCs (DHLA-AgNCs) are shown in Figure 5. The NCs can destroy the bacterial cells through similar mechanisms as the NPs because of the released ions and the increase in ROS.^{87,88, 89}

4.1 Activity of released ions

Bacterial biofilms are communities of one or more types of bacteria in a protective matrix that can grow on different surfaces. Conventional antibiotics such as vancomycin normally exhibit weak activity to remove the bacterial biofilms. However, the metal ions leached from AgNPs can penetrate the biofilms and kill the bacteria by interacting with the thiol groups of vital enzymes.⁹⁰ Because AgNCs are smaller than AgNPs, the AgNCs are much more susceptible to oxidative dissolution, facilitating the release of metal ions. This increased efficacy is balanced by the decreased stability of AgNCs relative to AgNPs.

The pH of the biofilm microenvironment (5.5–7) is substantially lower than physiological pH (7.35–7.45). Wu developed nanoantibiotics composed of self-assembled AgNCs with the pH-sensitive charge-reversal ligand poly(ethylene glycol)-poly(aminopropyl imidazoleaspartate)-polyalanine (PEG-PSB-PALA) called rAgNAs, and pH-insensitive poly(ethylene glycol)-poly(β -benzil-L-aspartate)-polyalanine (PEG-PIB-PALA) called uAgNAs (Figure 6). Under neutral physiological conditions, rAgNAs have high stability in the acidic microenvironment (pH 5.5) found in multidrug-resistant (MDR) bacterial biofilms. The protonation of imidazole groups from PEGPSB-PALA is triggered, facilitating local accumulation, retention, and the disassembly of rAgNAs to small AgNCs (Figure 6a). The AgNCs release Ag⁺ efficiently and rupture the bacterial membrane resulting in deep penetration into the biofilm. In contrast, uAgNAs show low penetration and accumulation in biofilm because of their pH-insensitivity.

The rAgNAs could reduce ~73% of the total bioburden of MDR biofilms (pH = 4.5–6.5), whereas vancomycin caused only an 8–25% reduction (Figure 6b). By the employment of a pH-responsive assembled-disassembled method, the released ions from NCs specifically attack the MDR biofilms, which will show immense therapeutic potential in the treatment of biofilm-related infectious diseases.

4.2 Elevation of ROS levels

ROS can be produced as a consequence of bacterial cellular metabolism, generating antibacterial effects. Various antimicrobials have been developed based on elevating the ROS level. In one study, the 6-mercaptohexanoic acid (MHA) protected AuNPs have insignificant antibacterial activities.⁶⁷ However, as the sizes of these Au nanomaterials decrease, intracellular ROS is significantly enhanced and dramatic metabolic imbalances occur in the bacterial cells.^{92–94} Likewise, 6-MHA protected AuNCs (1 nm) promote the upregulation of pro-oxidative enzymes while suppressing reductive enzymes, leading to much higher amounts of intracellular ROS compared to 6-MHA protected AuNPs (2 nm) (Figure 7).⁶⁷ The AuNCs show a half-maximal inhibitory concentration (IC₅₀) of ~2.6 μM, and ~3.2 μM for Gram-positive (*S. aureus*, *S. epidermidis*, *B. subtilis*), and Gram-negative bacteria (*E. coli*, *P. aeruginosa*), respectively.

4.3 Catalytic effects

The catalytic activities of nanomaterials are significantly influenced by their sizes/or the number of atoms present.⁹⁵ NCs with certain atomic numbers can be used as effective biocatalysts and can be indirectly used as antimicrobials by catalyzing specific processes. Some coinage metal NCs can act as artificial nano-sized catalysts/or enzymes (i.e. nanozymes) to enhance the antimicrobial activities of other intermediate agents.^{96, 97} For instance, CuNCs can mimic the activities of horseradish peroxidase and induce the generation of •OH radicals from H₂O₂ via decomposition of the O-O bonds of peroxides.^{98–100} Compared with horseradish peroxidase, CuNCs can have higher catalytic activity near-neutral pH.¹⁰¹ Miao et al. used papain-protected CuNCs (CuNCs@Papain) to catalyze the decomposition of H₂O₂ (Figure 8).¹⁰² After mixing the peroxide with CuNCs, •OH radicals were immediately formed. This product significantly enhanced the antibacterial activity of H₂O₂ against Gram-positive (*S. aureus*) and -negative bacteria (*E. coli*).^{103–106} The inhibition diameters of two types of bacteria (*M. luteus* and *P. aeruginosa*) treated with CuNCs and H₂O₂ are much larger than that treated with just CuNCs or H₂O₂, respectively (Figure 8A, B). We found that transition metal NPs can be efficient enzyme-mimic materials in the field of bacterial eradication.^{107–109} The smaller Au/Ag/Cu NCs show potential for antibacterial applications, based on their high catalytic.¹¹⁰ In one example, the group of Jiang used a mice skin infection model to show the ability of their quaternary ammonium-capped AuNCs (QA-AuNCs) to combat MRSA infections *in vivo*.⁷² At present, most studies have demonstrated antimicrobial efficacy of NC-based nanozymes *in vitro* and only a few studies have included *in vivo* models. More studies with *in vivo* models are required to evaluate the effect of nanozyme NCs not only on bacteria but also on the host.

4.4 Synergistic activity of NCs

4.4.1 NCs Combined with antibacterial agents—The use of coinage metal NCs in combination with traditional antibiotics or antibacterial methods can provide enhanced antibacterial effects.^{111–114} Different antibiotics have been used for the elimination of either or both Gram-positive and -negative bacteria.^{115–118, 119} Antimicrobial peptides (AMPs) have broad-spectrum antibacterial properties but are expensive to manufacture.^{120, 121} In one

study, bacitracin, a peptide antibiotic mainly used against Gram-positive bacteria, was integrated into metal NCs (AgNCs, AuNCs, and CuNCs) for enhancement of antibacterial efficiency.¹¹⁸ The inhibition diameter and MIC of *S. aureus* treated with AgNCs@Bacitracin, AuNCs@Bacitracin, CuNCs@Bacitracin, or bacitracin only were 0.82 cm and 6.25 $\mu\text{g}\cdot\text{mL}^{-1}$, 0.54 cm and 200 $\mu\text{g}\cdot\text{mL}^{-1}$, 0.62 cm and 50 $\mu\text{g}\cdot\text{mL}^{-1}$, 0.50 cm and 200 $\mu\text{g}\cdot\text{mL}^{-1}$, respectively. AgNCs@Bacitracin demonstrated to be the most robust, with mechanisms of action including membrane damage and ROS production. Chen et al. showed that functionalization of AuNCs with antimicrobial peptide surfactin (SFT) (SFT/DT-AuNCs) yielded higher antimicrobial efficiency for multiple types of bacteria, including one non-MDR Gram-positive (*S. aureus*), three non-MDR Gram-negative (*S. enteritidis*, *E. coli*, and *P. vulgaris*), and one MDR Gram-positive bacteria (MRSA), compared with SFT alone.¹⁴⁷ This enhanced antibacterial efficiency is ascribed to the greater disintegration of the bacterial membrane, leading to cytoplasmic leakage. The minimum inhibitory concentration (MIC) values of SFT/DT-AuNCs were more than 80-fold lower than that of SFT alone (Figure 9). By using AMP-combined AuNCs, the dosage can be significantly reduced, generating an antimicrobial with a better therapeutic window.

Deoxyribonuclease (DNase) is an enzyme that can degrade the extracellular DNA present in the EPS matrix of the biofilm, thereby weakening its structure and making it more penetrable by antimicrobials. By sensitizing NCs with light, i.e. phototherapy, NCs can convert light into heat or induce the generation of ROS.¹²³ Since the addition of near-infrared light (NIR) does enhance the antibacterial activity of DNase alone, NIR was used to activate the phototherapeutic effects of bovine serum albumin-protected AuNCs (BSA-AuNCs) for killing planktonic bacteria.¹²⁴ By combining NIR (808 nm, 2 W/cm², 10 min) with BSA-AuNCs, *S. aureus* and *P. aeruginosa* biofilms were partially removed. Xie et al developed deoxyribonuclease (DNase) functionalized (AuNCs) (DNase-AuNCs), which can be activated with NIR phototherapy (Figure 10). By the production of singlet oxygen (¹O₂) and elevation of the temperature, the system (NIR+DNase-AuNCs) can significantly enhance the removal efficiency of the MDR *S. aureus* (~80% biomass loss) and *P. aeruginosa* (~75% biomass loss) biofilms (Figure 10b). SEM characterization also suggests that bacteria in the biofilms are significantly damaged (Figure 10c). This study shows that a combination of NIR-activation with specific NCs provides a promising phototherapeutic strategy for treating biofilm infections.

Some photobactericidal agents such as crystal violet (CV) can be used to kill bacteria under white light illumination. However, CV is only antimicrobial when intense light is used. Hwang et al. encapsulated CV in a polymer for the eradication of *E. coli* and *S. aureus*, but no antibacterial activity was observed, either under dark or white light illumination (312 lux) (Figure 11).⁵⁹ However, cysteine-protected AuNCs (Au₂₅(Cys)₁₈) ‘activate’ CV to provide more effective photobactericidal agents under white light. Reactions were promoted by generating an electron transfer pathway from CV to the AuNCs. This enhanced the ROS (H₂O₂) level and bactericidal activity. The viability of *E. coli* and *S. aureus* significantly decreased by combining CV, AuNCs and white light with intensity from 200 to 429 lux. Such low illumination intensity is readily available at healthcare facilities.

Summarized in Table 2 are different combinations of traditional antibiotics and NCs. All the combined approaches demonstrated improved antibacterial activities compared to the antibiotic alone. This synergy indicates that NCs amplify the activity of antibiotics.

4.4.2 Combination with ineffective agents—Some agents do not show high antibacterial activity themselves but gain efficacy as NCs.^{4, 129, 130} High antibacterial efficiencies against *E. coli* have been found for AgNCs that were regulated by DNA templates (Figure 12). Three DNA functionalized AgNCs were synthesized by a one-pot method and developed as antimicrobials for the eradication of *E. coli*. By simply tuning the sequences of the DNA templates, the AgNCs can be engineered as emitters of different colors (Figure 12a). The blue emitters have lower antibacterial activities than the yellow emitters while the red emitters display the highest antibacterial activity (Figure 12b).¹³¹ The antibacterial activities of NCs can be tuned by small molecules as well. Tannic acids (TA) have insignificant antibacterial activities, but TA functionalized CuNCs (TA-CuNCs) provided an excellent activity for killing Gram-positive bacteria.⁴ Along with these functionalization, Wang encapsulated AgNCs in the sacrificial ELB (extract of Luria-Bertani (LB) broth medium) by light irradiation, resulting in a higher enhanced intracellular ROS generation.¹³² The sacrificial ELB vehicle and its synergistic effects with AgNCs increased the antibacterial activities of AgNCs by 3–4 orders of magnitude toward both Gram-positive (*S. aureus*, *B. subtilis*) and Gram-negative bacteria (*E. coli*, *P. aeruginosa*). The use of specific templates or coatings can result in the formation of AgNCs with appropriate surface conditions, structures or other properties, allowing the eradication of specific types of bacteria with high efficiencies.

Antimicrobial NCs can potentiate and/or impart antimicrobial effects when used in combination with other molecules. They can also be used as nanocatalysts for reactions that increase ROS levels. Overall, NCs offer multiple bactericidal strategies promising in combatting bacterial infections.

5 NCs as delivery vehicles for antimicrobials

Efficient delivery systems for antibacterial drugs can enhance their activity. Loading antibacterial drugs in coinage metal NCs has been explored as a strategy for eradication of bacteria, lessening off-target effects, and reducing the toxicity of the drugs. Zheng *et al.* loaded peptide-protected AuNCs (Au-SGaa, i.e., the pentapeptide γ -ECGDADA (GSHaa) coated AuNCs) with vancomycin.¹³⁴ The vancomycin is only released upon exposure to Gram-positive bacteria such as *S. aureus*, *B. subtilis* and *B. cereus* due to the stronger binding affinity of vancomycin with bacteria than that with AuNCs. Since the binding between the vancomycin-conjugated Au-SGaa (Au-SGaa-Van) and vancomycin are reversible, the vancomycin was released controllably. Au-SGaa-Van and vancomycin both show MIC of 2.0 $\mu\text{g mL}^{-1}$. On the other hand, none of the drugs decrease the viability of *E. coli* at any concentrations. Liu *et al.* generated cross-linked proteins using AuNCs (CP-GNC) by heating a mixture containing BSA, HAuNCs, and NaOH at 97 °C for 15 min.¹³⁵ The CP-GNC only attaches to bacterial cells at low pH (<5.2) and detaches from the bacterial cells at high pH (>7) (Figure 13). Based on pH-responsive properties, the CP-GNC can selectively serve as a pH controllable and recyclable tool for bacterial cell imaging

and antibiotic delivery for disinfecting bacteria. Only 0.02 mg mL^{-1} of the drugs such as ampicillin and chloramphenicol was required to inhibit the growth of *E. coli* cells (BL21-gold), whereas neither CP-GNC nor antibiotics alone have antibacterial effects at similar concentrations. This enhanced activity indicates NCs cannot only release the drug but also exhibit synergistically enhanced efficiency.

6 Biosafety of NCs

6.1 Toxicity

For materials to be successfully used as therapeutics, the toxicity and harmful effects to humans must be considered.^{31–33, 111} Some smaller NCs tend to release more ions than NPs, which contributes to both their antibacterial activity and host cytotoxicity.^{74, 136, 137} To get around this limitation, strategies have been employed to confine the released ions. For instance, in protein cultures, NCs can partially or completely sulfidate with strongly associated human serum albumin (HSA) coronas, significantly reducing toxicity.¹³⁸ Another work used acrylic resins to incorporate tetraoctylammonium functionalized DHLA-AgNCs (TOA-AgNCs) (Figure 14). TOA enables the TOA-AgNCs to possess positive charges and better antibacterial activity against Gram-negative (*Klebsiella pneumoniae*) and Gram-positive bacteria (*Staphylococcus aureus*) compared to the negatively charged DHLA-AgNCs. However, only a little silver ion is released from AgNCs because of the confinement of acrylic resins (Figure 14b). The colorimetric cell counting Kit-8 (CCK) assay shows that TOA-AgNCs at different concentrations ($<1 \text{ wt\%}$) do not influence the proliferation pattern of the NIH3T3 cells (Figure 14c). This indicates the low toxicity of the acrylic resins incorporated with TOA-AgNCs.

The high efficiency of NCs can minimize their toxicity to mammalian cells.¹³⁹ For instance, Tay et al. reported the high viability of NCM460 epithelial cells in the presence of different concentrations ($<10 \text{ }\mu\text{M}$) of glutathione (GSH) and 3-Mercaptopropionic acid (MPA) protected AuNCs.¹⁴⁰ Meanwhile, the low toxicity to different mammalian cells has been demonstrated by various other coinage metal NCs within certain concentration ranges (Table 3).

6.2 Drug resistance

Drug resistance is a challenge for standard antibiotics.¹⁴⁸ There are three main targets for traditional antibiotics: disruption of bacterial cell wall synthesis, interfering with translation during protein synthesis, and interrupting DNA replication processes. Resistance is developed against each of the above-mentioned targets.^{149–153} Many antibiotics are designed to target specific processes, but the bacteria can develop new cell processes that circumvent the action of the antibiotic. Overcoming these mechanisms, the coinage metal NCs can destroy bacteria through multiple processes including 1) disruption of the bacterial cell membrane, 2) bacterial cell wall penetration, 3) DNA and protein disruption. The simultaneous multiple attack pathways by NCs are challenging for bacteria to counter.

AuNCs can provide high antibacterial activity against MDR bacteria. Li et al. used a one-pot method to obtain water-soluble AuNCs protected by (11-mercaptoundecyl)-N,N,N-

trimethylammonium bromide (MUTAB) (MUTAB-AuNCs).¹⁵⁴ The MIC of the MUTAB-AuNCs (1 µg/mL) against a drug-resistant bacteria (VRE, vancomycin-resistant *Enterococci*) is much lower than antibiotics such as ampicillin, vancomycin, oxacillin, and linezolid that are all >100 µg/mL).⁷² AuNCs also damage MDR bacteria through functionalization with the antibiotic. For instance, the MRSA was not affected by β -lactam antibiotic ampicillin alone (Figure 15C).¹²⁶ Functionalization of AuNCs with this antibiotic, however, caused bacterial membrane rupture (Figure 15D).

The comparison of AuNCs to traditional antibiotics for combating resistance development is shown in Figure 16. *S. aureus* developed resistance against vancomycin within few days after a few exposures to a sublethal dose of vancomycin. However, no resistance was developed in the presence of 4,6-diamino-2-mercaptopyrimidine-protected AuNCs (DAMP-AuNCs) even after thirty days of multiple cycles (Figure 16a).¹⁵⁵ Xie et al. demonstrated that MDR Gram-positive bacteria did not develop the resistance toward QA-AuNCs, whereas resistance was observed within thirty days for penicillin (Figure 16b). Yi et al. found no drug resistance development towards MUTAB-AuNCs after 21 successful passages against *P. aeruginosa* (Figure 16c).¹⁵⁴

In stark contrast, obvious resistance of *P. aeruginosa* towards Ceftriaxone appeared in the fourth generation. These results suggest that AuNCs can be promising antimicrobial agents against bacteria without inducing drug resistance.

7 Promising antibacterial NC treatments

7.1 External applications

The relatively low cost of NCs coupled with their ease of production makes them practical for a wide range of applications. For instance, Xie et al modified orthodontic devices (e.g., Invisalign aligner) with QA-AuNCs (Figure 17).¹⁵⁶ The adhesion of cariogenic *Streptococcus mutans* and the formation of biofilms were efficiently inhibited by this modified aligner. These products were quite immunosilent: mice fed with QA-GNCs for 2 weeks showed no increase in inflammatory biomarkers including IL-6 and TNF- α (Figure 17b), and cells including WBC and LYM (Figure 17c). Our lab likewise used AuNCs (<100 µM) as efficient antibacterial foams for cleaning bacteria-contaminated surfaces such as knobs, tables, cell phones, and etc.¹⁵⁷ The foams show insignificant toxicity to mammalian cells, such as human umbilical vein cells (HUVECs) and mouse osteoblastic cells (MC3T3-E1). Based on price and efficacy, NCs are promising and safe antimicrobials for surface applications.

7.2 Internal applications

Metal accumulation is important for internally-administered drugs. For instance, antibacterial CuNPs were embedded in hydrogels for treating *S. aureus*-infected wounds with photothermal therapy.¹⁵⁸ However, these CuNPs could only be used as external dressings. NCs offer new targets compared with larger nanomaterials. Nair et al. found that AuNCs can pass the blood-brain barrier with appropriate functionalization, which is promising for treating brain diseases.¹⁵⁹ With fewer concerns regarding metal accumulation,

QA-AuNCs were used as intraperitoneal-injection drugs to heal the MRSA infected wounds of mice.⁷²

8 Efficient theranostic nanoplatforms

The fluorescent behavior of coinage metal NCs is distinct from that of non-fluorescent metallic NPs. Through fluorescence monitoring, the detection and disinfection of bacteria can be realized simultaneously.⁷² However, most coinage metal NCs are not efficient emitters. For instance, several groups have reported the two-photon emission from AuNCs, but this output is not observed under the light that is compatible with the biomedical environment.¹⁶⁰ To solve this problem, Vangara *et al.* attached DHLA-AuNCs (GNCs) to hexamethylenediamine functionalized graphene quantum dots (GQDs) (Figure 18).¹⁶¹ GQDs with two-photon absorption act as donors while AuNCs act as acceptors (Figure 18a). The luminescence of the combined hybrid (GQD-AuNC) under NIR excitation is significantly enhanced because of the fluorescence resonance energy transfer (FRET) mechanism.¹⁶² Figure 18b, c shows the two-photon emission from GOD-GNC for MRSA (embedded in a collagen matrix), which was observed from 75 μm and 50 μm depth, respectively. Meanwhile, AuNCs have long-lived triplet excited states, that can produce ROS ($^1\text{O}_2$). The GOD-GNC further enhanced the $^1\text{O}_2$ production killing 50% MRSA in the presence of NIR light (860 nm) after 10 min in the presence of AuNCs and GQDs separately, but the GQD-GNC killed almost 100% of the bacteria.

9 Prospects and Conclusions

Gold, silver, and copper NCs have great potential for combating bacterial infections and contamination with high efficiency. Currently, the antibacterial activities of these NCs are mainly attributed to elevated ROS levels and released ions. However, both the ROS and released ions that give NCs their bactericidal activity bring concerns of host toxicity. This lack of selectivity requires balancing of activity, which can be achieved by 'smart' materials. For instance, pH-responsive NCs only show toxicity in bacterial environments. The ease of functionalization of NCs can also be taken used to expand the therapeutic window of NCs. Extensive investigations are still needed to better understand the fate and effect of NCs on both bacteria and host. Most of the studies to date have looked at *in vitro* activity; *In vivo* demonstration of activity and safety will be required to optimize their applicability in clinical settings.

In summary, NCs are highly promising antibacterials. NCs provide 1) multiple mechanistic families of effective nanoantibiotics; 2) a new strategy for overcoming multidrug resistance; 3) useful treatments for biofilm infections; 4) increased efficiency for traditional antibiotics; and 5) useful carriers for antibiotic (drug) delivery. Taken together, these capabilities individually and collectively open new doors for antimicrobial treatments that will be critical for addressing the emerging threat of MDR bacteria.

Acknowledgments

This work was supported by the NIH (AI134770) and National Natural Science Foundation of China (No. 81702105 and 21701069).

Notes and references

1. Reichart TM, Uzarski JR and Mello CM, *Analyst*, 2019, 144, 7242–7249. [PubMed: 31687669]
2. Li B and Webster TJ, *J Orthop Res*, 2018, 36, 22–32. [PubMed: 28722231]
3. Smith PA, Koehler MFT, Girgis HS, Yan D, Chen Y, Chen Y, Crawford JJ, Durk MR, Higuchi RI, Kang J, Murray J, Paraselli P, Park S, Phung W, Quinn JG, Roberts TC, Rouge L, Schwarz JB, Skippington E, Wai J, Xu M, Yu Z, Zhang H, Tan MW and Heise CE, *Nature*, 2018, 561, 189–194. [PubMed: 30209367]
4. Xia J, Wang W, Hai X, E S, Shu Y and Wang J, *Chinese Chemical Letters*, 2019, 30, 421–424.
5. Samara E, Moriarty TF, Decosterd LA, Richards RG, Gautier E and Wahl P, *Bone Joint Res*, 2017, 6, 296–306. [PubMed: 28515059]
6. Gross M, *Current Biology*, 2019, 29, R859–R861.
7. Ben Y, Fu C, Hu M, Liu L, Wong MH and Zheng C, *Environ Res*, 2019, 169, 483–493. [PubMed: 30530088]
8. Silver SBSKYLL, *Biochemical pharmacology*, 2017, 133, 63–73. [PubMed: 28087253]
9. Stephan NCNCJVLKS, *Drug Discovery Today*, 2017, 22 234–248. [PubMed: 27890668]
10. Yong-Guan Zhu YZ, Li Bing, Huang Chu-Long, Zhang Si-Yu, Yu Shen, Chen Yong-Shan, Zhang Tong, Gillings Michael R., Su Jian-Qiang, *Nature microbiology*, 2017, 2, 16270
11. Silverman SM, Moses JE and Sharpless KB, *Chemistry*, 2017, 23, 79–83. [PubMed: 27747932]
12. McEwen SA, Angulo FJ, Collignon PJ and Conly JM, *The Lancet Planetary Health*, 2018, 2, e279–e282. [PubMed: 30074886]
13. Gupta A, Mumtaz S, Li C-H, Hussain I and Rotello VM, *Chemical Society Reviews*, 2018, DOI: 10.1039/c7cs00748e, in press.
14. Das S and Dhar BB, *RSC Adv*, 2014, 4, 46285–46292.
15. Silvero CM, Rocca DM, de la Villarmois EA, Fournier K, Lanterna AE, Perez MF, Becerra MC and Scaiano JC, *ACS Omega*, 2018, 3, 1220–1230. [PubMed: 30023798]
16. Wadhwani P, Heidenreich N, Podeyn B, Burck J and Ulrich AS, *Biomater Sci*, 2017, 5, 817–827. [PubMed: 28275774]
17. Lee B, Park J, Ryu M, Kim S, Joo M, Yeom JH, Kim S, Park Y, Lee K and Bae J, *Sci Rep*, 2017, 7, 13572. [PubMed: 29051620]
18. Pan W, Zhao J and Chen Q, *J Agric Food Chem*, 2015, 63, 8068–8074. [PubMed: 26308972]
19. Liu X, Marrakchi M, Xu D, Dong H and Andreescu S, *Biosens Bioelectron*, 2016, 80, 9–16. [PubMed: 26802747]
20. Chang MS, Yoo JH, Woo DH and Chun MS, *Analyst*, 2015, 140, 7997–8006. [PubMed: 26524182]
21. Ye R, Zhu C, Song Y, Song J, Fu S, Lu Q, Yang X, Zhu MJ, Du D, Li H and Lin Y, *Nanoscale*, 2016, 8, 18980–18986. [PubMed: 27808326]
22. Surwade P, Ghildyal C, Weikel C, Luxton T, Peloquin D, Fan X and Shah V, *J Antibiot (Tokyo)*, 2019, 72, 50–53. [PubMed: 30361634]
23. Chen J, Yang L, Chen J, Liu W, Zhang D, Xu P, Dai T, Shang L, Yang Y, Tang S, Zhang Y, Lin H, Chen Z and Huang M, *Chemical Engineering Journal*, 2019, 374, 1373–1381.
24. Tsai TT, Huang TH, Chang CJ, Yi-Ju Ho N, Tseng YT and Chen CF, *Sci Rep*, 2017, 7, 3155. [PubMed: 28600506]
25. Rai A, Prabhune A and Perry CC, *Journal of Materials Chemistry*, 2010, 20, 6789.
26. Song XR, Goswami N, Yang HH and Xie J, *Analyst*, 2016, 141, 3126–3140. [PubMed: 27146244]
27. Wang H, Ho KT, Scheckel KG, Wu F, Cantwell MG, Katz DR, Horowitz DB, Boothman WS and Burgess RM, *Environmental Science & Technology*, 2014, 48, 13711–13717. [PubMed: 25369427]
28. Wang N, Wei X, Zheng AQ, Yang T, Chen ML and Wang JH, *ACS Sens*, 2017, 2, 371–378. [PubMed: 28723213]

29. Duncan B, Le NDB, Alexander C, Gupta A, Yesilbag Tonga G, Yazdani M, Landis RF, Wang LS, Yan B, Burmaoglu S, Li X and Rotello VM, *ACS Nano*, 2017, 11, 5339–5343. [PubMed: 28423269]
30. Maier L, Pruteanu M, Kuhn M, Zeller G, Telzerow A, Anderson EE, Brochado AR, Fernandez KC, Dose H, Mori H, Patil KR, Bork P and Typas A, *Nature*, 2018, 555, 623–628. [PubMed: 29555994]
31. Tan X and Jin R, *Wiley Interdiscip Rev Nanomed Nanobiotechnol*, 2013, 5, 569–581. [PubMed: 23939885]
32. Gao Li RJ, *Acc. Chem. Res.*, 2013, 46, 1749–1758. [PubMed: 23534692]
33. Wang Y, De S and Yan N, *Chem Commun (Camb)*, 2016, 52, 6210–6224. [PubMed: 27022992]
34. Chen LY, Wang CW, Yuan Z and Chang HT, *Anal Chem*, 2015, 87, 216–229. [PubMed: 25275676]
35. Zhang L and Wang E, *Nano Today*, 2014, 9, 132–157.
36. Jin R, Zeng C, Zhou M and Chen Y, *Chem Rev*, 2016, 116, 10346–10413. [PubMed: 27585252]
37. Mao B, Qu F, Zhu S and You J, *Sensors and Actuators B: Chemical*, 2016, 234, 338–344.
38. Xiaoning Li SMR, Gupta Akash, Saha Krishnendu, Jiang Ziwen, Moyano Daniel F., Sahar Ali, Riley Margaret A., and Rotello Vincent M., *ACS Nano*, 2014, 8, 10682–10686. [PubMed: 25232643]
39. Jiang X, Du B, Huang Y and Zheng J, *Nano Today*, 2018, 21, 106–125. [PubMed: 31327979]
40. Jin R, *Nanoscale*, 2015, 7, 1549–1565. [PubMed: 25532730]
41. Dipankar Bain SM, Paramanik Bipattaran, Patra Amitava, *ACS Sustainable Chem. Eng.*, 2018, 6, 2334–2343.
42. Wu Z and Jin R, *Nano Lett*, 2010, 10, 2568–2573. [PubMed: 20550101]
43. Chen Y, Yang T, Pan H, Yuan Y, Chen L, Liu M, Zhang K, Zhang S, Wu P and Xu J, *J Am Chem Soc*, 2014, 136, 1686–1689. [PubMed: 24437963]
44. Muhammad VN Farooq U, Rozhkova Elena A., *Sci Rep*, 2018, 8, 2907. [PubMed: 29440698]
45. Alexandropoulos DI, Fournet A, Cunha-Silva L, Christou G and Stamatatos TC, *Inorg Chem*, 2016, 55, 12118–12121. [PubMed: 27934429]
46. Joya KS, Sinatra L, AbdulHalim LG, Joshi CP, Hedhili MN, Bakr OM and Hussain I, *Nanoscale*, 2016, 8, 9695–9703. [PubMed: 27109550]
47. Guo Z, Xiao C, Maligal-Ganesh RV, Zhou L, Goh TW, Li X, Tesfagaber D, Thiel A and Huang W, *ACS Catalysis*, 2014, 4, 1340–1348.
48. Xie X, Sun J, Cao B and Duan H, *RSC Advances*, 2017, 7, 4933–4940.
49. Tang M, Zhang J, Yang C, Zheng Y and Jiang H, *Front Chem*, 2020, 8, 181. [PubMed: 32266210]
50. Li D, Chen Z, Wan Z, Yang T, Wang H and Mei X, *RSC Advances*, 2016, 6, 34090–34095.
51. Markus IABarthel J, Petrelli Alessia, Avellini Tommaso, Scarpellini Alice, Bertoni Giovanni, Armirotti Andrea, Moreels Iwan, Pellegrino Teresa, *ACS Nano*, 2015, 9, 11886–11897. [PubMed: 26512975]
52. Zhou C, Long M, Qin Y, Sun X and Zheng J, *Angew Chem Int Ed Engl*, 2011, 50, 3168–3172. [PubMed: 21374769]
53. Sultan D, Ye D, Heo GS, Zhang X, Luehmann H, Yue Y, Detering L, Komarov S, Taylor S, Tai YC, Rubin JB, Chen H and Liu Y, *Small*, 2018, 14, e1703115. [PubMed: 29966035]
54. Nair LV, Nair RV, Shenoy SJ, Thekkuveetil A and Jayasree RS, *Journal of Materials Chemistry B*, 2017, 5, 8314–8321. [PubMed: 32264500]
55. Zhang XD, Wu D, Shen X, Liu PX, Fan FY and Fan SJ, *Biomaterials*, 2012, 33, 4628–4638. [PubMed: 22459191]
56. Zhao Y, Sultan D, Detering L, Luehmann H and Liu Y, *Nanoscale*, 2014, 6, 13501–13509. [PubMed: 25266128]
57. Porret E, Le Guevel X and Coll JL, *J Mater Chem B*, 2020, DOI: 10.1039/c9tb02767j, in press.
58. Gao G, Chen R, He M, Li J, Li J, Wang L and Sun T, *Biomaterials*, 2019, 194, 36–46. [PubMed: 30576972]

59. Hwang GB, Huang H, Wu G, Shin J, Kafizas A, Karu K, Toit HD, Alotaibi AM, Mohammad-Hadi L, Allan E, MacRobert AJ, Gavriilidis A and Parkin IP, *Nat Commun*, 2020, 11, 1207. [PubMed: 32139700]
60. Liu Y, Wang S, Wang Z, Yao Q, Fang S, Zhou X, Yuan X and Xie J, *J Mater Chem B*, 2020, DOI: 10.1039/d0tb00073f, in press.
61. Li D, Kumari B, Makabenta JM, Gupta A and Rotello V, *Nanoscale*, 2019, 11, 22172–22181. [PubMed: 31746916]
62. Siamak Javani RL, Latorre Alfonso, Flors Cristina, Cortajarena Aitziber L., and Somoza Álvaro, *ACS Appl. Mater. Interfaces*, 2016, 8, 10147–10154. [PubMed: 27058628]
63. Wang HY, Hua XW, Wu FG, Li B, Liu P, Gu N, Wang Z and Chen Z, *ACS Appl Mater Interfaces*, 2015, 7, 7082–7092. [PubMed: 25785786]
64. Mishra SK, Raveendran S, Ferreira JM and Kannan S, *Langmuir*, 2016, 32, 10305–10316. [PubMed: 27685160]
65. Baghdasaryan A, Grillo R, Roy Bhattacharya S, Sharma M, Reginato E, Theraulaz H, Dolamic I, Dadras M, Rudaz S, Varesio E and Burgi T, *ACS Applied Nano Materials*, 2018, 1, 4258–4267.
66. Li D, Wang G, Peng Y, Chen Z, Gao X, Cheng L and Mei X, *Nanoscale Advances*, 2019, 1, 1086–1095.
67. Zheng K, Setyawati MI, Leong DT and Xie J, *ACS Nano*, 2017, 11, 6904–6910. [PubMed: 28595000]
68. Feng ZV, Gunsolus IL, Qiu TA, Hurley KR, Nyberg LH, Frew H, Johnson KP, Vartanian AM, Jacob LM, Lohse SE, Torelli MD, Hamers RJ, Murphy CJ and Haynes CL, *Chem Sci*, 2015, 6, 5186–5196. [PubMed: 29449924]
69. Wang D, Xu J, Yang J and Zhou S, *J Colloid Interface Sci*, 2020, 563, 261–271. [PubMed: 31884250]
70. Liu F, He D, Yu Y, Cheng L and Zhang S, *Bioconjug Chem*, 2019, 30, 541–546. [PubMed: 30726061]
71. Huda S, Smoukov SK, Nakanishi H, Kowalczyk B, Bishop K and Grzybowski BA, *ACS Appl Mater Interfaces*, 2010, 2, 1206–1210. [PubMed: 20423140]
72. Xie Y, Liu Y, Yang J, Liu Y, Hu F, Zhu K and Jiang X, *Angew Chem Int Ed Engl*, 2018, 57, 3958–3962. [PubMed: 29423995]
73. Cotton GC, Gee C, Jude A, Duncan WJ, Abdelmoneim D and Coates DE, *RSC Advances*, 2019, 9, 6973–6985.
74. Jin JC, Wu XJ, Xu J, Wang BB, Jiang FL and Liu Y, *Biomater Sci*, 2017, 5, 247–257. [PubMed: 27921105]
75. Pallavicini P, Taglietti A, Dacarro G, Diaz-Fernandez YA, Galli M, Grisoli P, Patrini M, Santucci De Magistris G and Zanoni R, *J Colloid Interface Sci*, 2010, 350, 110–116. [PubMed: 20621306]
76. Taglietti A, Diaz Fernandez YA, Amato E, Cucca L, Dacarro G, Grisoli P, Necchi V, Pallavicini P, Pasotti L and Patrini M, *Langmuir*, 2012, 28, 8140–8148. [PubMed: 22546237]
77. Gao S, Zhu Y, Wang J, Zhang F, Li J and Jin J, *Advanced Functional Materials*, 2018, DOI: 10.1002/adfm.201801944, 1801944.
78. Javani S, Lorca R, Latorre A, Flors C, Cortajarena AL and Somoza A, *ACS Appl Mater Interfaces*, 2016, 8, 10147–10154. [PubMed: 27058628]
79. Yuan X, Setyawati MI, Tan AS, Ong CN, Leong DT and Xie J, *NPG Asia Materials*, 2013, 5, e39–e39.
80. Yuan X, Setyawati MI, Leong DT and Xie J, *Nano Research*, 2013, 7, 301–307.
81. Shareena Dasari TP, Zhang Y and Yu H, *Biochem Pharmacol (Los Angel)*, 2015, 4, 1000199.
82. Huang Z, He K, Song Z, Zeng G, Chen A, Yuan L, Li H, Hu L, Guo Z and Chen G, *Chemosphere*, 2018, 211, 573–583. [PubMed: 30092538]
83. Huma Z.-e., Gupta A, Javed I, Das R, Hussain SZ, Mumtaz S, Hussain I and Rotello VM, *ACS Omega*, 2018, 3, 16721–16727. [PubMed: 30613808]
84. Du C, Yan H, Liang J, Luo A, Wang L, Zhu J, Xiong H and Chen Y, *Int J Nanomedicine*, 2017, 12, 8599–8613. [PubMed: 29238194]
85. Zheng K, Setyawati MI, Leong DT and Xie J, *Chemistry of Materials*, 2018, 30, 2800–2808.

86. Palza H, Delgado K and Pinochet I, *Journal of Applied Polymer Science*, 2015, 132, 41232.
87. Mathew MS, Davis J and Joseph K, *Analyst*, 2018, 143, 3841–3849. [PubMed: 29999047]
88. Chen K, Xie K, Long Q, Deng L, Fu Z, Xiao H and Xie L, *RSC Advances*, 2017, 7, 29368–29377.
89. Kim QLFTN, Kim JO, *Journal of Materials Science: Materials in Medicine*, 1998, 9, 129–134. [PubMed: 15348901]
90. Singh N, Paknikar KM and Rajwade J, *Environmental Science: Nano*, 2019, 6, 1812–1828.
91. Wu J, Li F, Hu X, Lu J, Sun X, Gao J and Ling D, *ACS Central Science*, 2019, 5, 1366–1376. [PubMed: 31482119]
92. Pranantyo D, Liu P, Zhong W, Kang ET and Chan-Park MB, *Biomacromolecules*, 2019, DOI: 10.1021/acs.biomac.9b00392, 2922–2933. [PubMed: 31305998]
93. Agnihotri S, Mukherji S and Mukherji S, *RSC Adv*, 2014, 4, 3974–3983.
94. Chang T-K, Cheng T-M, Chu H-L, Tan S-H, Kuo J-C, Hsu P-H, Su C-Y, Chen H-M, Lee C-M and Kuo T-R, *ACS Sustainable Chemistry & Engineering*, 2019, 7, 15479–15486.
95. Du X and Jin R, *ACS Nano*, 2019, 13, 7383–7387. [PubMed: 31246403]
96. Cao-Milan R, He LD, Shorkey S, Tonga GY, Wang LS, Zhang X, Uddin I, Das R, Sulak M and Rotello VM, *Mol Syst Des Eng*, 2017, 2, 624–628. [PubMed: 29430303]
97. Liu L and Corma A, *Chem Rev*, 2018, DOI: 10.1021/acs.chemrev.7b00776, in press.
98. Bucekova M, Buriova M, Pekarik L, Majtan V and Majtan J, *Scientific Reports*, 2018, 8, 9016. [PubMed: 29899358]
99. Ge J, Dong Z-Z, Bai D-M, Zhang L, Hu Y-L, Ji D-Y and Li Z-H, *New Journal of Chemistry*, 2017, 41, 9718–9723.
100. Gligorovski S, Strekowski R, Barbati S and Vione D, *Chem Rev*, 2015, 115, 13051–13092. [PubMed: 26630000]
101. Hu L, Yuan Y, Zhang L, Zhao J, Majeed S and Xu G, *Anal Chim Acta*, 2013, 762, 83–86. [PubMed: 23327949]
102. Miao H, Zhong D, Zhou Z and Yang X, *Nanoscale*, 2015, 7, 19066–19072. [PubMed: 26514845]
103. Chakraborty S, Babanova S, Rocha RC, Desireddy A, Artyushkova K, Boncella AE, Atanassov P and Martinez JS, *J Am Chem Soc*, 2015, 137, 11678–11687. [PubMed: 26288369]
104. Xu S, Wang Y, Zhou D, Kuang M, Fang D, Yang W, Wei S and Ma L, *Sci Rep*, 2016, 6, 39157. [PubMed: 27966650]
105. Ghosh C, Mondal T and Bhattacharyya K, *J Colloid Interface Sci*, 2017, 494, 74–81. [PubMed: 28135630]
106. Meng F, Yin H, Li Y, Zheng S, Gan F and Ye G, *Microchemical Journal*, 2018, 141, 431–437.
107. Gupta A, Das R, Yesilbag Tonga G, Mizuhara T and Rotello VM, *ACS Nano*, 2018, 12, 89–94. [PubMed: 29244484]
108. Mumtaz S, Wang LS, Hussain SZ, Abdullah M, Huma Z, Iqbal Z, Creran B, Rotello VM and Hussain I, *Chem Commun (Camb)*, 2017, 53, 12306–12308. [PubMed: 29094116]
109. Chen J, Jackson AA, Rotello VM and Nugen SR, *Small*, 2016, 12, 2469–2475. [PubMed: 26997252]
110. Basu K, Paul S, Jana R, Datta A and Banerjee A, *ACS Sustainable Chemistry & Engineering*, 2018, 7, 1998–2007.
111. Hussain S, Joo J, Kang J, Kim B, Braun GB, She ZG, Kim D, Mann AP, Molder T, Teesalu T, Carnazza S, Guglielmino S, Sailor MJ and Ruoslahti E, *Nat Biomed Eng*, 2018, 2, 95–103. [PubMed: 29955439]
112. Wright GD, *Trends Microbiol*, 2016, 24, 862–871. [PubMed: 27430191]
113. Zhu S, Wang X, Li S, Liu L and Li L, *ACS Appl Mater Interfaces*, 2020, DOI: 10.1021/acsami.0c00310, in press.
114. Girija A, Ravindran, Balasubramanian S, Bright R, Cowin AJ, Goswami N and Vasilev K, *ChemNanoMat*, 2019, 5, 1176–1181.
115. Hagrais M, Mohammad H, Mandour MS, Hegazy YA, Ghiaty A, Seleem MN and Mayhoub AS, *J Med Chem*, 2017, 60, 4074–4085. [PubMed: 28436655]

116. Venkateswarlu Yarlagadda SS, Paramanandham Krishnamoorthy, Shome Bibek R., Haldar Jayanta, *Angewandte Chemie*, 2015, 54, 13644–13649. [PubMed: 26473305]
117. Gardete S and Tomasz A, *J Clin Invest*, 2014, 124, 2836–2840. [PubMed: 24983424]
118. Wang S, Wang Y, Peng Y and Yang X, *ACS Appl Mater Interfaces*, 2019, 11, 8461–8469. [PubMed: 30714708]
119. Shi H, Zou J, Zhang T, Che H, Gao X, Wang C, Wang Y and Xue C, *J Agric Food Chem*, 2018, 66, 475–484. [PubMed: 29254330]
120. Tucker AT, Leonard SP, DuBois CD, Knauf GA, Cunningham AL, Wilke CO, Trent MS and Davies BW, *Cell*, 2018, 172, 618–628 e613. [PubMed: 29307492]
121. Ageitos JM, Sanchez-Perez A, Calo-Mata P and Villa TG, *Biochem Pharmacol*, 2017, 133, 117–138. [PubMed: 27663838]
122. Chen W-Y, Chang H-Y, Lu J-K, Huang Y-C, Harroun SG, Tseng Y-T, Li Y-J, Huang C-C and Chang H-T, *Advanced Functional Materials*, 2015, 25, 7189–7199.
123. Yang D, Gulzar A, Yang G, Gai S, He F, Dai Y, Zhong C and Yang P, *Small*, 2017, 13, 1703007.
124. Xie Y, Zheng W and Jiang X, *ACS Appl Mater Interfaces*, 2020, DOI: 10.1021/acsami.9b21777, in press.
125. Prada YA, Guzman F, Ortiz C, Cabanzo R, Torres R and Mejia-Ospino E, *Protein J*, 2019, 38, 506–514. [PubMed: 31119600]
126. Kalita S, Kandimalla R, Bhowal AC, Kotoky J and Kundu S, *Sci Rep*, 2018, 8, 5778. [PubMed: 29636496]
127. Lara HH, Black DM, Moon C, Orr E, Lopez P, Alvarez MM, Baghdasarian G, Lopez-Ribot J and Whetten RL, *ACS Omega*, 2019, 4, 21914–21920. [PubMed: 31891070]
128. Weiyu Chen JL, Chen Weijen, Luo Liyang, Guang Eric Wei, Yu Diau, Chen Chie, *Nanomedicine*, 2010, 5, 755–764. [PubMed: 20662646]
129. Higaki T, Li Q, Zhou M, Zhao S, Li Y, Li S and Jin R, *Acc Chem Res*, 2018, 51, 2764–2773. [PubMed: 30372028]
130. Nain A, Tseng YT, Wei SC, Periasamy AP, Huang CC, Tseng FG and Chang HT, *J Hazard Mater*, 2020, 389, 121821. [PubMed: 31879116]
131. Yang L, Yao C, Li F, Dong Y, Zhang Z and Yang D, *Small*, 2018, 14, e1800185. [PubMed: 29575604]
132. Wang Z, Fang Y, Zhou X, Li Z, Zhu H, Du F, Yuan X, Yao Q and Xie J, *Nano Research*, 2020, 13, 203–208.
133. Siamak Javani RL, Latorre Alfonso, Flors Cristina, Cortajarena Aitziber, Somoza Álvaro, *ACS Appl. Mater. Interfaces* 2016, 8, 10147–10154. [PubMed: 27058628]
134. Li Q, Pan Y, Chen T, Du Y, Ge H, Zhang B, Xie J, Yu H and Zhu M, *Nanoscale*, 2018, 10, 10166–10172. [PubMed: 29786738]
135. Liu P, Wang H, Hiltunen JK, Chen Z and Shen J, *Particle & Particle Systems Characterization*, 2015, 32, 749–755.
136. Dobias J and Bernier-Latmani R, *Environmental Science & Technology*, 2013, 47, 4140–4146. [PubMed: 23517230]
137. Tominaga C, Shitomi K, Miyaji H and Kawasaki H, *ACS Applied Nano Materials*, 2018, 1, 4809–4818.
138. Shang L, Brandholt S, Stockmar F, Trouillet V, Bruns M and Nienhaus GU, *Small*, 2012, 8, 661–665. [PubMed: 22213653]
139. Gong L, Wang Y and Liu J, *Biomater Sci*, 2017, 5, 1393–1406. [PubMed: 28484751]
140. Tay CY, Yu Y, Setyawati MI, Xie J and Leong DT, *Nano Research*, 2014, 7, 805–815.
141. Liang G, Jin X, Zhang S and Xing D, *Biomaterials*, 2017, 144, 95–104. [PubMed: 28834765]
142. Yang Y, Wang S, Xu C, Xie A, Shen Y and Zhu M, *Chem Commun (Camb)*, 2018, 54, 2731–2734. [PubMed: 29479592]
143. Xiaojuan Wang YW, He Hua, Ma Xiqi, Chen Qi, Zhang Shuai, Ge Baosheng, Wang Shengjie, Nau Werner M., and Huang Fang, *ACS Appl. Mater. Interfaces.*, 2017, 9, 17799–17806. [PubMed: 28492304]

144. Kundu N, Mukherjee D, Maiti TK and Sarkar N, *J Phys Chem Lett*, 2017, 8, 2291–2297. [PubMed: 28468496]
145. Wang X, Wang N, Li L, Xiao R, Yuan L, Yang X and Li N, *RSC Advances*, 2017, 7, 32536–32542.
146. Ghosh R, Sahoo AK, Ghosh SS, Paul A and Chattopadhyay A, *ACS Appl Mater Interfaces*, 2014, 6, 3822–3828. [PubMed: 24571718]
147. Santiago-Gonzalez B, Monguzzi A, Caputo M, Villa C, Prato M, Santambrogio C, Torrente Y, Meinardi F and Brovelli S, *Sci Rep*, 2017, 7, 5976. [PubMed: 28729689]
148. Furusawa C, Horinouchi T and Maeda T, *Curr Opin Biotechnol*, 2018, 54, 45–49. [PubMed: 29452927]
149. Aslam B, Wang W, Arshad MI, Khurshid M, Muzammil S, Rasool MH, Nisar MA, Alvi RF, Aslam MA, Qamar MU, Salamat MKF and Baloch Z, *Infect Drug Resist*, 2018, 11, 1645–1658. [PubMed: 30349322]
150. Poole K, *Journal of Applied Microbiology Symposium Supplement* 2002, 92, 55S–64S.
151. Brooks BD and Brooks AE, *Adv Drug Deliv Rev*, 2014, 78, 14–27. [PubMed: 25450262]
152. He Y, Yuan Q, Mathieu J, Stadler L, Senéhi N, Sun R and Alvarez PJJ, *npj Clean Water*, 2020, 3.
153. Witzky A, Tollerson R 2nd and Ibba M, *Open Biol*, 2019, 9, 190051. [PubMed: 31288624]
154. Li Y, Zhen J, Tian Q, Shen C, Zhang L, Yang K and Shang L, *J Colloid Interface Sci*, 2020, 569, 235–243. [PubMed: 32113021]
155. Zheng Y, Liu W, Qin Z, Chen Y, Jiang H and Wang X, *Bioconjug Chem*, 2018, 29, 3094–3103. [PubMed: 30063328]
156. Xie Y, Zhang M, Zhang W, Liu X, Zheng W and Jiang X, *ACS Biomaterials Science & Engineering*, 2020, 6, 1239–1246.
157. Pengfei Zhuang KL, Qian Kun, Li Dan, Mei Xifan, *IET Nanobiotechnology*, 2020, DOI: 10.1049/iet-nbt.2019.0356, in press.
158. Tao B, Lin C, Deng Y, Yuan Z, Shen X, Chen M, He Y, Peng Z, Hu Y and Cai K, *J Mater Chem B*, 2019, 7, 2534–2548. [PubMed: 32255130]
159. Nair LV, Nair RV, Shenoy SJ, Thekkuveetil A and Jayasree RS, *J Mater Chem B*, 2017, 5, 8314–8321. [PubMed: 32264500]
160. Olesiak-Banska J, Waszkielewicz M, Obstarczyk P and Samoc M, *Chem Soc Rev*, 2019, 48, 4087–4117. [PubMed: 31292567]
161. Vangara A, Pramanik A, Gao Y, Gates K, Begum S and Chandra Ray P, *ACS Applied Bio Materials*, 2018, 1, 298–309.
162. Li D, Chen Z and Mei X, *Adv Colloid Interface Sci*, 2017, 250, 25–39. [PubMed: 29132640]

Figure 1.

UV-Vis absorption spectra (a, a1), fluorescence excitation (EX) and emission (EM) spectra (b, b1), typical TEM image (c, c1) and statistic size distribution (d, d1) of green (a-d) and red (a1-d1) fluorescent CuNCs (inset a and a1, photograph of the samples under a 365 nm UV lamp). Reproduced from ref.⁶⁶ with permission from The Royal Society of Chemistry.

Figure 2.
The possible advantages of using coinage metal NCs as antimicrobials compared to NPs.
The crystal structure of typical AuNCs was adapted from ref.⁶⁷

Figure 3.

One-step fabrication of GSH-AuNCs and QA-AuNCs in water solutions at 70 C°.

Glutathione (GSH) was used as the reductant and QA was employed as the protecting ligand. All reagents were put into a single pot and the products were directly obtained and used for antibacterial applications. QA: N,N,N-trimethyl-(11-mercaptaundecyl) ammonium chloride. Adapted with permission from ref.⁷² with slight modifications Copyright 2018 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

Figure 4.

Damaged (a) and intact (b) membranes of bacteria interacting with negatively charged and zwitterionic AuNCs. (c) Zeta potential (mV) of different ligand-protected Au₂₅NCs in water. (d-f) Percentage of dead *S. aureus* after treatment with ligand-protected Au₂₅NCs for 2 h. The AuNCs were protected by 6-mercaptopentanoic acid (MHA), 3-mercaptopentanoic acid (MBA), L-cysteine (Cys), cysteamine hydrochloride (Cystm), 2-mercaptoethanol (MetH), or the combination of these ligands, respectively. Higher % D dead bacteria were observed for more negative AuNCs. Reproduced from ref.⁸⁵ with permission from American Chemical Society Copyright 2018.

Figure 5.

Mechanisms of antibacterial activity of DHLA-AgNCs against *E. coli*: (a) damage the outer membrane and permeate into *E. coli* DH 5 α cells; (b) diffuse into the *E. coli* DSM 4230 cells through porin channels; (c) damage the respiratory chain in the cytoplasmic membrane; (d) release of Ag⁺; (e) and (f) the released Ag⁺ interacts with DNA in the cytoplasm; (g) The released Ag⁺ interacts with the respiratory chain. Reproduced from ref.⁷⁴ with permission from The Royal Society of Chemistry.

Figure 6.

(a) Schematic illustration of the design and construction of rAgNAs and therapeutic mechanisms. (b) Total bioburden of the MDR biofilm after treatment. (c) Viability of the biofilm bacteria after treatment ($n = 4$). Reproduced from ref.⁹¹ with permission from American Chemical Society, Copyright 2019.

Figure 7.

(a) AuNCs can affect the cellular functions of *S. aureus* by inducing intracellular ROS production. (b) Comparison of AuNCs and AuNPs in eradicating bacteria. Reproduced from ref.⁶⁷ with permission from American Chemical Society, Copyright 2017.

Figure 8.

(A) Inhibition diameters of CuNCs plus H₂O₂ against *M. luteus* and *P. aeruginosa*, respectively; (B) the calculate relative average diameters. (C) Schematic illustration of the synthesis of CuNCs and their application as antibacterial agents. Reproduced from ref.¹⁰² with permission from The Royal Society of Chemistry.

Figure 9.

Comparison of MICs (in terms of the concentration of SFT) of SFT, SFT_{0.05}/DT-AuNDs, SFT_{0.1}/DT-AuNDs, SFT_{0.25}/DT-Au NDs, SFT_{0.5}/DT-Au NDs, and SFT_{1.0}/DT-Au NDs against five bacteria (*E. coli*, *P. vulgaris*, MRSA, *S. aureus*, and *S. enteritidis*). Error bars represent the standard deviation of three repeated measurements. Adapted with permission from ref.¹²² Copyright 2015 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

Figure 10.

(a) Scheme of NIR-light activated phototherapy by AuNCs for dispersing biofilms. (b) The reduction of biofilm biomass after different treatments, including saline (control), NIR-only, DNase-only (200 $\mu\text{g/mL}$), BSA-AuNCs-only (200 $\mu\text{g/mL}$), DNase-AuNCs-only (200 $\mu\text{g/mL}$), or DNase plus NIR (NIR+DNase), BSA-AuNCs plus NIR (NIR+BSA-AuNCs), DNase-AuNCs (200 $\mu\text{g/mL}$) plus NIR (NIR+DNase-AuNCs). (c) MDR *S. aureus* and MDR *P. aeruginosa* formed biofilms on the Invisalign aligners after treatment. Reproduced from ref.¹²⁴ Copyright 2020 American Chemical Society.

Figure 11.

Bactericidal activity of control (Con), $[\text{Au}_{25}(\text{Cys})_{18}]$ only (AuNCs), CV only, Cysteine only (Cys) and, the combined CV& $[\text{Au}_{25}(\text{Cys})_{18}]$ (CV-Au) against *S. aureus* (a, b) and *E. coli* (c, d) in the dark for 6 (a) and 24 hours (c), and in white light for 6 (b) and 24 hours (d).

CFU/mL indicates colony forming unit per mL. Blue star ($<10^2$ CFU mL $^{-1}$). Adapted with permission from ref.⁵⁹ Copyright 2020 Springer Nature.

Figure 12.

(a) Fabrication of different AgNC emitters and (b) the increase of the antibacterial activity against *E. coli* as the AgNC emitters change. (c) Kirby-Bauer disk diffusion susceptibility test. (A) Water, (B) AgNCs-1, (C) AgNCs-2, and (D) AgNCs-3. The results show that AgNCs-3 have the best antibacterial effect. Reproduced from ref. 133 Copyright 2016 American Chemical Society.

Figure 13. Schematic representation of cross-linked proteins with AuNCs (CP-GNCs) as a pH-sensitive tool for bioimaging and drug delivery. Reproduced from ref.¹³⁵ with permission from Copyright 2015 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

(a)

Double Antimicrobial Action of AgNCs and TOA Cation

(b)

(c)

Viability of NIH3T3 cells

Figure 14.

(a) Schematic for the antibacterial activity of TOA-AgNCs; (b) Amount of silver released by the antimicrobial acrylic resins (incorporating 0.1 wt%, 0.3 wt%, 1 wt%, and 3 wt% TOA-AgNCs) into 1 mL of water after a 24-h incubation; (c) Cytotoxic effects on NIH3T3 cells of acrylic resins incorporating various amounts of TOA-AgNCs (0, 0.1, 0.3, and 1 wt %) at 1, 3, and 5 day time points ($n = 4$, mean $\pm$ standard deviation). The absorbance at 450 nm is proportional to the NIH3T3 cell (immortalized mouse embryonic fibroblast cell line) viability. Reproduced from ref.¹³⁷ with permission from the American Chemical Society, Copyright 2018.

Figure 15.

Surface morphology of MRSA under FE-SEM upon treatment with PBS (A), AUNC-L (B), Free-Amp (C), and AUNC-L-Amp (D). Reproduced from ref.¹²⁶ with permission from Springer Nature Copyright 2018. Note: AUNC-L, lysozyme capped AuNCs; Free-Amp, β -lactam antibiotic ampicillin; AUNC-L-Amp, β -lactam antibiotic ampicillin functionalized AUNC-L.

Figure 16.

(a) Development of drug resistance towards DAMP-AuNCs and vancomycin by *S. aureus* upon multiple sublethal dose exposures;¹⁵⁵ Copyright 2018 American Chemical Society; (b) Development of drug resistance towards QA-AuNCs and oxacillin. Adapted with permission from ref.⁷² Copyright 2018 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim. (c) Drug resistance of *P. aeruginosa* towards MUTAB-AuNCs and ceftriaxone. Adapted with permission from ref.¹⁵⁴ Copyright 2020 Elsevier.

Figure 17.

(a) The oral toxicity of QA-AuNCs (depicted as GNCs in the figure) ($100 \mu\text{g/mL}$, $100 \mu\text{L}$) on the mouse feeding model for 2 weeks. (b) The inflammation index of mice in the feeding and control group. IL-6: interleukin-6 and TNF- α : tumor necrosis factor. (c) Cell numbers of white blood cells (WBC) and lymphocytes (LYM) in the mouse feeding model. Reproduced from ref.¹⁵⁶ with permission from the American Chemical Society, Copyright 2020. The inflammatory biomarkers all show negligible changes.

Figure 18.

(a) FRET-based two-photon excited theranostic nanoplatform using AuNCs attached to GQDs for two-photon imaging and photodynamic therapy (PDT) killing of MRSA. Two-photon luminescence image of MRSA attached nanoplatform at different depths at 75 μm (b) and 50 μm (c). (d) Comparison of the MRSA killing efficiency using 860 nm NIR light without nanoplatform (Con); NIR light with only AuNCs; NIR light with only GQDs and NIR light GQDs attached GNCs (GQD-GNC) based nanoplatform. All the reported data is for 10 min of exposure to NIR light (860 nm). Reproduced from ref.¹⁶¹ with permission from the American Chemical Society, Copyright 2018.

Table 1Synthesis of AgNCs and AgNPs for eradication of *E. coli*

NCs	Synthesis	Size	MIC	Ref.
DHLA-AgNPs	Functionalization of AgNPs by DHLA in organic solvents	~6 nm	2.5 µg/mL	73
DHLA-AgNCs	One-pot synthesis in water solution at room temperature	~2 nm	10 µg mL ⁻¹	74
GSH-AgNPs	Functionalization of AgNPs by GSH using dipping method	~7 nm	15 µg/mL	75, 76
GSH-AgNCs	Direct synthesis by cyclic NaBH ₄ reduction-decomposition method	<1.5 nm	100 µM	73

MIC: minimum inhibitory concentration

Table 2

The enhancement of antibacterial activity against multidrug-resistant pathogens by combining NCs with traditional antibiotics

Target	Drugs	MIC/IC ₅₀	Mechanism for enhancement	Ref.
MRSA	NBC2254	~73 μ M		
	AuNC@NBC2254	~4.7 μM	improves the interaction with the bacterial target	
	NBC2253	~6.7 μ M		125
	AuNC@NBC2253	~3.0 μM	improves the interaction with the bacterial target	
	Amp	ineffective		
Biofilm	AUNC-L-Amp	16 μg/mL	Improves the binding to the bacterial surface and enhanced permeation	126
	penicillin	140 μ M		
	Penicillin-AgNCs	2.3 μM	A synergetics effect for penicillins (inhibit the cell wall synthesis) and AgNCs (cell wall disruption and lysis)	127
PDRAB	Lysozyme	>10 μ g/mL		128
	Lysozyme-AuNCs	<2μg/mL	multivalent interactions enhance the interaction efficiency	

NBC2253: peptide (CGIYRSLKLIKSLVLIK); NBC2254: peptide (CALKLTAKRLVRKIGF); AuNC-L: lysozyme capped AuNCs; Amp: β -lactam antibiotic ampicillin ; Biofilm: *S. aureus* biofilm; penicillin: 6-aminopenicillanic acid; PDRAB: Pan-drug-resistant *Acinetobacter baumannii*. Both MIC and IC₅₀ were compared at the same conditions.

Table 3

Toxicity evaluation for the typical NCs

NCs	Cell culture	Effect	Ref.
RGD-AuNCs	HeLa, MCF-7, Hepatic L02	Not toxic for the cells with 120 μ M AuNCs	141
AuNCs@GTMS-FA	MCF-7, CHO	No noticeable toxicity at 800 μ g/mL under laser irradiation after 6 h	142
KCK-AuNCs	HT1080	90% cell viability after treatment with 500 μ g/mL for more than 12 h	143
GO-AgNCs	K562	Remarkable cell viability after 48 h (G0<100 μ g/mL; AgNCs< 4 μ g/mL)	144
GSH-AgNCs	MDCK	Not toxic (< 8 mg/mL) in the 4 h transportation assays	145
Lysozyme-CuNCs	HeLa,	Little cytotoxicity at 345 μ g/mL	146
Ag/Au-NCs	NIH/3T3	Prolonged anti-cytotoxic activity at 7 μ M	147

Note: GTMS-FA, gelatin (G), folic acid (FA), two-level mesoporous silica (TMS); RGD, cyclic arginine-glycine-aspartic acid (c(RGDyC)) peptide; KCK, tripeptide Lys–Cys–Lys; GO-AgNCs, graphene oxide (GO) assembled AgNCs; Ag/Au-NC, The the Ag clusters with a thin mantle of aggregated Au-thiolate complexes capped with 16-mercaptohexadecanoic acid (MHDA)-tetrabutylammonium (TBA) salt.