Recent outbreaks of bacterial infection leading to human fatalities have been a motivational force for us to develop antibacterial agents with high potency and long-term stability. A novel cobalt (Co) based metal-organic framework (MOF) was tested and shown to be highly effective at inactivating model microorganisms. Gram-negative bacteria, *Escherichia coli* (strains DH5alpha and XL1-Blue) were selected to determine the antibacterial activities of the Co MOF. In this MOF, the Co serves as a central element and an octa-topic carboxylate ligand, tetrakis [(3,5-dicarboxyphenyl)-oxamethyl] methane (TDM8) serves as a bridging linker. X-ray crystallographic studies indicate that Co-TDM crystallizes in tetragonal space group *P*4 212 with a porous 3D framework. The potency of the Co-TDM disinfectant was evaluated using a minimal bactericidal concentration (MBC) benchmark and was determined to be 10–15 ppm within a short incubation time period (<60 min). Compared with previous work using silver nanoparticles and silver-modified TiO2 nano-composites over the same time period, the MBC and effectiveness of Co-TDM are superior. Electron microscopy images indicate that the Co-TDM displayed distinctive grain boundaries and well-developed reticulates. The Co active sites rapidly catalyzed the lipid peroxidation, causing rupture of the bacterial membrane followed by inactivation, with 100% recycling and high persistence (>4 weeks). This MOF-based approach may lead to a new paradigm for MOF applications in diverse biological fields due to their inherent porous structure, tunable surface functional groups, and adjustable metal coordination environments.

1. Introduction

The recent outbreak of a fatal strain of enterohemorrhagic *Escherichia coli* (E. Coli) in Lower Saxony, Germany in May and June of 2011 has demonstrated the importance of clean water and the strategies involved in purification and disinfection. Traditionally, disinfection treatments have utilized numerous chemical agents,[1] such as the use of phenol[2] and ethanol, mainly for disinfection of hands[3] and surfaces,[4] and sodium hypochlorite (bleach)[5] for potable water disinfection. The main drawback of chemical disinfectants is product breakdown[6] and lack of long-term stability.[7]

This has led to the development of ozone treatment[8] and the use of ultraviolet or gamma irradiation,[9] which are easier to apply, but still gave rise to by-products, some of which are toxic.[10,11]

Apart from chemical disinfectants or irradiation/ozone treatment, the use of silver metal has recently drawn increased attention.[12] Silver is effective but expensive, therefore its use is limited in the disinfection of water.[13] It has re-emerged as the principal disinfection agent against
Gram-negative/Gram-positive microbes, particularly when coated onto a core of titania (TiO₂). This technique has permitted economically viable use of silver, keeping costs down due to the use of silver/titania nanoparticles, which afford high surface area, low dosage and long-term stability. In this study, metal-organic frameworks (MOFs), which also contain structured metal atoms similar to Ag/TiO₂ nanoparticles, were shown to inactivate E. coli effectively. MOFs have been widely explored in applications such as small molecule capture, drug delivery, imaging, and catalysis, but have rarely been studied as a disinfectant. The putative mechanism for inactivation, with potential for high potency and long-term stability, is via rupture of the lipid membrane, causing cell death. These MOF materials are crystalline compounds in which metal ions or clusters are coordinated to organic ligands forming an interconnected pore system. The difficulty of designing and preparing solid-state materials with pre-designated structures for specific applications once posed a great challenge in materials science. After two decades of exploration, it is now routine to design and control a MOF structure precisely via tuning of the inorganic and organic components, which are readily available due to the power of organic synthesis. One salient feature of MOFs is their crystallinity, which allows facile determination of their crystal structures, facilitating adjustments to the original design. Via this powerful strategy, various molecular architectures (polygonal rings, polyhedral cages, and polymeric structures) can be pre-designed and their fixed geometry and cooperative stability can be tuned via modification of ligands. Through functionalization of the organic linkers, MOFs can be designed to suit their end applications. Although significant amount of research on porous MOFs has been reported, most of these materials are based on di-topic, tri-topic, tetra-topic, or hexa-topic carbonylate ligands. In this research, an octa-topic carbonylate ligand has been conceived and prepared. Applications of MOFs are highly diversified due to the unique nature of their tunable porosity, high surface area and structural diversity, and include shape/size/enantio-selective catalysis and gas storage and separation for next generation alternative energy solutions. Nanoscale metal-organic frameworks for biomedical imaging and drug delivery have recently been reported by Lin and co-workers. Application of bimetallic MOFs in an emerging concept called “theragnostics” has been investigated by Liu and Palakurthi. Bactericidal activity of copper MOFs has also been recently observed. The overarching goal of this research focuses on the development and application of new materials for the betterment of public health, exemplified by use of MOFs as disinfectants in drinking water. Here, the antimicrobial activity of the MOF is measured, using the minimum bacterial concentration (MBC) as a standard benchmark to determine efficacy of the disinfectant. The unique porous structure, multifunctionalizing radicals, and tunable properties, as discussed above for novel MOFs, contribute to its bactericidal efficacy against E. coli, which is compared against literature values for other class of disinfectants.

In this paper, a new Co-based metal-organic framework (CoTDM) was synthesized using an octa-topic carboxylate ligand, tetrakis[3,5-dicarboxyphenyl]oxamethyl] methane acid (abbreviated as H₄TDM) applying a facile hydro-solvothermal method. The Co-TDM was demonstrated as a viable disinfectant with high potency (10–15 ppm) toward inactivation of the Gram-negative bacteria, E. Coli, specifically the DH5alpha and XL1-Blue strains. Structural characterization of the grown single crystals was performed using instrumental techniques including X-ray crystallography and transmission and scanning electron microscopy (T/SEM). The main results reported in this work focus on: (a) Synthesis of the novel Co-TDM MOF via a bottom-up hydro-solvo thermal method with optimized fabrication parameters; (b) Analysis of textural and crystalline structure and elemental composition using microscopic and spectroscopic techniques; and (c) Application of Co-TDM as a potent bacterial disinfectant. The originality of this work is two-fold: 1) fabrication of a novel Co-based MOF with octa-topic ligands, and 2) documented ultra-low minimal bactericidal concentration (MBC) against Gram-negative microbes with high efficacy and persistence. This approach to using specific metal centers bound within an organic framework for disinfection of microbes provides a potential new method to mitigate bacterial contamination in food and water, which represents a severe threat to public health and disease control.

2. Results and Discussion

2.1. Fabrication Optimization

The tetrakis[3,5-dicarboxyphenyl]oxamethyl]methane acid (H₄TDM) ligand (Figure 1a) was synthesized in two steps (substitution and hydrolysis), following a similar procedure to that

![Figure 1](image-url)

**Figure 1.** Fabrication of Co-TDM via a bottom-up solvothermal method and its biological application to inactivating bacteria, a) Molecular structure of the ligand tetrakis [3,5-dicarboxyphenyl]oxamethyl] methane acid, H₄TDM; b) TEM images of intact E. coli (left-side), schematic of interaction between bacteria and Co-TDM (middle), and damaged E. coli (right-side).
previously reported.\textsuperscript{12} Briefly, the generation process of Co-TDM through a hydro-solvothermal approach aims to produce well-defined and highly crystallized coordinative compounds. This octa-topic TDM\textsuperscript{8} ligand serves as the organic linker coordinating with the Co central element. A mixture composed of dimethylformamide (DMF) with a small portion of de-ionized water was used, which acted as both solvent and stabilizing agent. Pink crystals with a molecular formula of [Co\textsubscript{4}((H\textsubscript{2}O))\textsubscript{4}(TDM)(H\textsubscript{2}O)\textsubscript{4}] were formed. The nucleation of Co-TDM crystal units was believed to be promoted when the solvent molecules were evaporated from the solution. This rapid nucleation favors the formation of Co-TDM with controllable size and monodispersity, as shown in the electron microscopy analyses. Because a complex can be formed between the ligand anions (TDM\textsuperscript{8}−) and cobalt metal cations (Co\textsuperscript{2+}), the central particle growth is inhibited indeterminately and can be terminated at a designated size. The TDM\textsuperscript{8}− ligand regulates the size and stability of Co-TDM crystals.

The liquid phase of the hydro-solvothermal fabrication is a key step in the synthesis process, as it allows for more rapid diffusion of reactants (by many orders of magnitude) than in the solid phase. Due to the fast kinetics of the nucleation reaction, the synthesis can be performed at lower temperatures (85°C) than are commonly used, with shorter treatment times (24 h) and at ambient atmospheric pressure. The complex formed from the metal ions and the functional groups of the ligands via coordinated covalent bonds allow the important tunable porosity and geometry of the single crystals to be obtained through control of the molecular size. We speculate that the metal cation, Co\textsuperscript{2+}, acts as a chemically active site to catalyze the peroxidation of lipid membrane, weakening the membrane and leading to lysis and inactivation of the microbe(Figure 1b).

2.2. Crystalline Structure of Co-TDM

The structure of [Co\textsubscript{4}((H\textsubscript{2}O))\textsubscript{4}(TDM)(H\textsubscript{2}O)\textsubscript{4}] was determined by X-ray crystallography, and was found to be the tetragonal space group P4\textsubscript{2}2\textsubscript{1}m (a = 20.078(4) Å, c = 11.252 (2) Å). The Co-TDM adopts a distorted dimeric H\textsubscript{2}O-centered basic carboxylate cluster [Co\textsubscript{2}((H\textsubscript{2}O))(O\textsubscript{2}CR)\textsubscript{4}(H\textsubscript{2}O)\textsubscript{4}] (Figure 2) as its secondary building unit (SBU). The coordination geometry of each cobalt is octahedral. Every TDM\textsuperscript{8}− is connected to eight cobalt centers, acting as an octa-topic ligand (Figure 2). The pairs of Co\textsubscript{2} clusters are bridged by the carboxylate groups from two different TDM\textsuperscript{8}− ligands and one H\textsubscript{2}O molecule to form a 3-dimensional porous framework (Figure 3a). Along the c axis, there exist dumbbell-shaped open channels of 11 × 6 Å. To obtain an in-depth understanding of the Co-TDM structure, topological analysis was performed by defining [Co\textsubscript{4}((H\textsubscript{2}O))\textsubscript{4}(O\textsubscript{2}CR)\textsubscript{4}(H\textsubscript{2}O)\textsubscript{4}] SBU as four-connected nodes and the quaternary carbon atoms of TDM\textsuperscript{8}− as eight-connected nodes. An unusual (4, 8)-connected scu type\textsuperscript{13} net\textsuperscript{14} of \{4\textsuperscript{16},6\textsuperscript{2}\} \{4\textsuperscript{6},6\textsuperscript{2}\} (Figure 3b).

2.3. Morphological Structure of Co-TDM and Bacteria

Both high-resolution transmission and scanning electron microscopy (TEM and SEM) were employed to study the fine structure of the crystals and their effect on bacterial membrane integrity. The microscopy results indicated that the bacteria were heavily damaged upon addition of the Co single crystals to the cell culture media.

2.3.1. Transmission Electron Microscopic Analyses of Co-TDM and Bacteria

To understand the morphological changes in the bacteria upon using Co-TDM as a disinfectant, a high-resolution transmission electron microscope equipped with X-ray energy dispersive spectroscopy (EDS or EDAX) and electron energy loss spectroscopy (EELS) analytical tools was employed (Figures 4a–d). The results (Figure 4a: intact DH5\textalpha) indicate that intact bacteria have a distinctive outer membrane and well-defined flagella, suggesting that the E. coli cell structure was well-preserved even under high vacuum and subjected to the high energy electron beam. E. coli co-incubated with Co-TDM lost their cellular cohesion (Figure 4a: damaged DH5\textalpha), with their outer membranes being heavily damaged or destroyed. TEM morphological

Figure 2. The connection mode of the TDM\textsuperscript{8}− anion and cobalt cations displaying polyhedral geometry; Co-TDM crystallizes in tetragonal space group P4\textsubscript{2}2\textsubscript{1}m with a porous 3D framework; hydrogen atoms are not shown for simplification; the inset shows the dimeric H\textsubscript{2}O-centered basic carboxylate cluster [Co\textsubscript{2}((H\textsubscript{2}O))(O\textsubscript{2}CR)\textsubscript{4}(H\textsubscript{2}O)\textsubscript{4}] (carbon, grey; oxygen, red; cobalt, magenta).

Figure 3. a) The 3-dimensional framework of a Co-TDM single crystal and the dumbbell-shaped open channels in the c direction, Co-centers (magenta) are shown in their polyhedral geometries; b) Schematic representation of the topology of Co-TDM, in which the [Co((H\textsubscript{2}O))\textsubscript{4}(O\textsubscript{2}CR)\textsubscript{4}(H\textsubscript{2}O)\textsubscript{4}] SBU acts as four-connected nodes (magenta) and quaternary carbon atoms of TDM\textsuperscript{8}− act as eight-connected nodes (green).
analyses also showed that the integrity of the XL1-Blue strain control (Figure 4b: intact XL1-Blue) was similarly maintained under high-energy electron beams and high vacuum, but the treated XL1-blue bacteria (Figure 4b: damaged XL1-Blue) could barely maintain their cell walls.

The above figures (Figure 4a and Figure 4b) indicates extensive blebbing (disintegration) of the membrane, condensation of the nuclear material (region 2) and membrane lysis into the extracellular matrix (region 3), however there is no indication of incorporation into the cytoplasm (region 1), suggesting the mode of reaction was through interaction with the outer wall/membrane. Similar effects were observed for both strains; the degree of damage for the XL1-Blue (LacIq strain) appeared to be more extensive under the same treatment conditions.

These two strains of *E. coli* (DH5α and XL1-Blue) were treated using ultralow doses of Co-TDM (10–15 ppm). Since there is a high utilization of the Co active sites, larger quantities, as would be required for chemical disinfectants (as demonstrated in a previous study) were not necessary.\(^{[35]}\) Co-TDM is very effective due to its large reactive surface area and unique chemical reactivity, which can inactivate bacteria with only a short co-incubation period (<60 min) and with long-term efficacy. It also was found that Co-TDM was very stable even under high energy electron beams. The tetragonal structure of the Co-TDM single crystals was very well maintained and no structural collapse was observed.

The full utilization of Co at the surface of the samples enhanced the bactericidal effects. Copper was detected and attributed to use of Cu-grids to support the Co-TDM samples in the TEM study. Upon incubation with Co-TDM, the elements inside and outside (Figure 4c and Figure 4d) the *E. coli* cell structure consisted of cations: sodium, potassium, calcium (Ca); and anions: nitrogen, sulfur, phosphorus, chlorine. K leakage is known to occur upon disruption of cellular respiration,\(^{[35]}\) and
is thus an indicator of membrane damage. The other heteroatoms are found in proteins (DNA building blocks), indicating inactivation through rupturing of the bacterial membrane; it has been shown that Co interacts strongly with membranes containing glycerophosphoryl moieties.\[36\] Membrane lysis results in the release of the bacteria’s contents into surrounding media, leading to the cell death. These observations correspond with the TEM morphological images (Figure 4b), suggesting that membrane damage of *E. coli* upon addition of the Co-TDM crystals is the major cause of inactivation. The mode of action appears to be six-fold and will be discussed in Section 2.6.

2.3.2. EELS Elemental Mapping of the Intact and Damaged Bacteria

To understand the extent of damage to the *E. coli* cell wall, the elemental composition was mapped using EELS (Figure 5a and Figure 5b). The leakage of cations and anions towards the surface can be readily visualized through EELS results. The elemental map of the intact bacteria clearly depicts multi-atomic elements distributed within the cell membrane (Figure 5a); no leakage was detected. Upon addition of Co-TDM, the integrity of the bacteria has failed; the heavy damage to the cell wall is

![EELS elemental mapping analyses](image)

**Figure 5.** EELS elemental mapping analyses. a) *E. coli* DH5α and XL1-Blue controls, showing selected elements from the intact DH5α: multi-atomic elements are evenly distributed inside the cell membrane, no leakage detected. b) Destroyed DH5α and XL1-Blue cells post Co-TDM inoculation (Note: data for DH5α were selected for illustration: the membrane structure was completely destroyed, resulting in lysis and expulsion of nuclear and cytoplasmic components), showing selected elements from the damaged DH5α; the multi-atomic elements are randomly distributed inside and outside of cell membrane (note that no distinguishable membrane remains).
seen from the random distribution of the elements throughout the media (Figure 5b, selected elements (K, Na, N, and Cl) shown as indicators of membrane collapse). Evidently, the Co-TDM directly interacts with the cell wall of *E. coli*, causing severe cellular damage. At complete disintegration, it is impossible to distinguish the boundary of bacteria and the incubation media, indicating complete collapse of the cell wall and depolarization of the membrane. It is hypothesized that the inducer (e.g., the chemical oxidant, antibiotic or transition metal oxide nanoparticle or in our case the Co-TDM) can cause ion channel formation resulting in depolarization of the cell membrane. This depolarization would result in monovalent ion transport across the membrane and increased acidity, which has been observed in the literature.[37] Furthermore, since the pH difference (across the cell membrane) is partially sustained by the H\(^+\) pump, if the pumps were active, addition of the inducer would not lead to an immediate drop in the difference in potential across the cell membrane ($\Delta$V). This sustained pH difference can be attributed to an increase in the concentration difference of protons across the cell membrane ($\Delta$H). In other words, the inhibitory effects of the inducer can be partially offset by increased output of the H\(^+\) pump, keeping the $\Delta$ pH values high to compensate for membrane depolarization until the concentration of the inducer becomes inhibitory. Two phenomenological events would then be expected: a) a pH-dependence of the inducer. Assuming the inducer is cobalt, it has been shown that inhibition/stimulation toward lipid peroxidation (and presumably therefore membrane depolarization) is pH-dependent[38] and b) concentration-dependence, such that above certain thresholds the difference in the membrane pH ($\Delta$ pH) is insufficient to maintain membrane polarization. This concentration dependence was also observed in our study (data not shown) and other studies with cobalt salts and could also partly explain the “blebbing” observed in the TEM images of *E. coli* inhibited with Co-TDM. What is unequivocal is that the Co-TDM leads to membrane depolarization and transport of inorganic monovalent cations, cytotoxicity, and cellular inactivation. The mode of inactivation may involve several integrated steps summarized and discussed in Section 2.6 (see Figure 8). The mode of inactivation may involve in several steps such as: i) weakening of the cell wall, ii) through lipid peroxidation; (ii) cross-linking of protein-to-DNA leading to inhibition of certain DNA repair mechanisms; iv) disruption of ion transport via membrane insertion; v) cation uptake in preference to Fe\(^{2+}\) resulting in disruption of scaffold-bound clusters and iron-sulfur enzymes resulting in destabilization of iron homeostasis by degrading the iron-sulfur cluster/sulfur mobilization (ISC/SUF) regulatory pathways resulting in toxicity and inactivation; and vi) disruption of the membrane potential difference through membrane insertion or formation of ion channels. Any metal center capable of disruption of the iron balance, such as Co\(^{2+}\), Ag\(^{+}\) or Cu\(^{+}\), would presumably be toxic, which recently was demonstrated for copper.[39]

### 2.3.3. Scanning Electron Microscopy Analyses of Co-TDM

The surface morphology of the Co-TDM (Figures 6a–Figure 6d) indicated that well-defined and highly crystalline metal-organic frameworks were formed. The crystals displayed distinctive cubic geometry, with the areas averaging approximately 2.8×2.5×1.2 μm\(^2\). These domains provided a significant advantage for peroxidation of the bacterial cell membrane when the *E. coli* were treated using the Co-TDM. From the images (in Figure 6a to Figure 6c), it can be seen that the single crystals are highly stable under electron beam conditions. In general, the grain boundary of the Co-TDM can also be easily distinguished, which favors peroxidation of the cell membrane, since at the bulk level, we believe peroxidation may occur from these boundaries. The EDS analyses indicated the Co cation metal occurred at approximately $L_\alpha$ 0.776 keV and $K_\alpha$ 6.929 keV (Figure 6d), respectively. In tandem with the X-ray analysis, the highly crystalline and well-defined MOFs are also inherently pure in formulation, which was confirmed by the EDS data. It was also seen that the Co-TDM is highly porous and Co element occupied a specific location within the frameworks, according to the EDS mapping results. This ensures good reproducibility and low deviance in the operability of the Co-TDM measurement when used as disinfectants, since each crystal provides ultrahigh surface area and high active sites for peroxidation of the membrane at the molecular level. The Co-TDM derived by one-step hydro-solvothermal syntheses under mild conditions displayed a high purity crystalline fraction. The synthetic reproducibility with high product purity/structure is essential to evaluating the dependence of proposed applications on the tunable structure on MOFs.

### 2.4. Effectiveness of Bactericidal Performance of Co-TDM

To evaluate the bactericidal activity using Co-TDM, two commercially available strains of *E. coli* (DH5α and XLI-Blue) were used as model microorganisms. The methodology has previously been described.[40] Briefly, in control experiments, the antibacterial activity of pure ligands and Co(NO\(_3\))\(_2\) showed little or no antibacterial activity towards *E. coli* cells; zero or near zero percent of *E. coli* cells were inactivated at concentrations of 0.01-0.015 mg/mL (10-15 ppm). The antibacterial efficacy of Co-TDM was assessed by incubating *E. coli* for different time windows (Figure 7a and Figure 7b). Every hour, the absorbance of *E. coli* was collected by ultraviolet-visible scan (300–800 nm) and optical density (UV-Vis; OD\(_{600 \text{ nm}}\)) spectroscopy modes and compared to that with no bacteria (blank). It was found the absorbance was negligible, suggesting no growth of bacteria under these operational conditions; indicating Co-TDM exhibits highly efficient antibacterial performance, reducing the population to zero or near-zero. The minimal bactericidal concentration (MBC) ranging from 0.5-0.75 mg/50 mL (10-15 ppm) with rapid inactivation of *E. coli* was achieved shorter incubation time than 1 hr. A possible reason for this phenomenon is that Co-TDM has unique structure with octa-topic ligand, which serve as the reservoir for the metal ions when interact with the bacteria cell membrane. The large porosity, unique chemical reactivity, and high utilization of Co surface element also contribute to the high potency of antibacterial activities of the Co-TDM. The extent of Co\(^{2+}\) exposed to MOF motif’s surface increases and thus, the interaction probability between Co and the bacteria cell wall increases. Herein, the MOF moiety enables to achieve rapid antibacterial effectiveness. Additionally, the
Co-TDM prepared using two solvents showed similar results, suggesting the nature of solvents have negligible effects on the bactericidal activity.

2.5. Comparison of Bactericidal Performance Using Co-TDM and Other Materials

To evaluate the antimicrobial efficacy of the Co-TDM, a series of experiments were implemented using commonly used chemical disinfectant, silver nanoparticles, and nanocomposite Ag-doped titania (TiO$_2$). From the comparison (Figure 7c), it can be concluded that the Co-TDM displayed an intermediate MBC (10–15 ppm) within a short treatment period (less than 60 min), while the Ag nanoparticles required a long incubation time (>2 h, <10 ppm) with significant long-term stability.$^{41}$ The Ag-TiO$_2$ nanocomposite also displayed long-term but slow antibacterial effects (<10 ppm, 2 h) with the smallest MBC.$^{42}$ On the other hand, previously documented chemical treatments (hypochlorite and phenol) exhibit instantaneous disinfection, which decreased with time with hypochlorite due to oxidation.$^{35}$ It is critical to point out that both Co-TDM and nanomaterials showed persistent antibacterial performance under various treatment conditions; whereas, hypochlorite
Figure 7. Efficacy evaluation of Co-TDM as a disinfectant to inactivate E. coli. a) Absorbance as a function of wavelength: DH5α was selected (XL1-blue shows essentially equivalent data, data not shown); b) the absorbance as a function of treatment time: DH5α was selected (XL1-blue shows essentially equivalent data, data not shown). c) Comparison of bactericidal performance using Co-TDM, nanomaterials, and commonly used chemical disinfectants. Note all other comparative data was unused replicate data from a previous study. [40]
did not exhibit long-term persistence. From the comparison results (Supporting Information, Table 2), it can be concluded that the Co-TDM showed a rapid bactericidal effect with 100% recycling of the engineered composite. The nanoparticles also have significant effects over the long term. Although chemical disinfectants have been commonly used over the last century\(^{[43]}\) as the “first wave” and provide almost instantaneous efficacy against bacteria, their short-term stability limits their long-term effectiveness. These short-comings have been addressed with new engineered nanoparticles/composites as the “second wave” of putative disinfectants\(^{[44]}\) focusing on nanoparticles either as biofilms, sols or coatings as 1D/2D structures. Other 3D arrays in the form of structured lattices or zeolite-like frameworks were also shown to be partially effective as antibacterial agents with MBCs in the range of 500–1000 ppm (Supporting Information, Table 2).\(^{[45]}\) The use of 3D structured arrays has recently been updated through use of metal-organic frameworks for a variety of applications\(^{[46]}\) including the first application as a potential disinfectant by the Liu group\(^{[40]}\) and described in this study as octa-topic carboxylic substituted frameworks. The measured MBC of 15 ppm (current study) is an order of magnitude lower than use of organoboron framework (MBCs of 307 ppm, Supporting Information Table 1) as an alternative approach to engineered nanoparticles. The advantages of supramolecular heterocyclics are the same as observed with structured nanoparticles, with the additional benefits of size selectivity as observed with other macrocycles such as cyclodextrins,\(^{[47]}\) crown ethers, and cryptands;\(^{[48]}\) systems with the tunability of metal ion as a redox centre, affinity probe or other optical, physical parameter. This approach has several advantages, namely utilization of cobalt as the divalent cation, since divalent cobalt, unlike atomic cobalt, is toxic to microbes, presumably through one of the six mechanisms described previously, including possible competition with iron cations and inhibition of de novo iron-sulfur protein synthesis through inhibition of critical enzymes and shutting down of critical metabolic pathways.

2.6. Mechanistic Study of Bactericidal Activity

Our current and previous studies on antibacterial activity using a series of materials suggested that an integrated mechanism is applicable for bacteria inactivation, although the membrane damage of \(E.\ coli\) upon addition of the biocidal agents is the major cause. The synergistic effect of antimicrobial action was observed and appeared to be six-fold, (1) Diffusion-directed lipid-oxidation, (2) Direct interaction; (3) Reaction-oxygen species generation, (4) Cation transport interruption, (5) Chelation effects, and (6) Membrane depolarization (Figure 8). (see Section 2.3.2.).

(1) Diffusion-directed lipid-oxidation: In this mechanism of bactericidal activity using Co-TDM, the diffusion of the MOF motifs in the media will be a dominant step, along a chemical gradient 90 degrees to the plane of the cell wall within 5-4500 seconds, depending on the particle size and diffusion coefficient. The distance between the initial particles and cell wall can be described by the following equation:

\[
x^2 = 2Dt
\]

where \(x\) is the average value of the distance between the initial particles and cell wall, 100 < \(x\) <300 \(\mu\)m, which is estimated from experimental data; \(D\) is the diffusion coefficient, \(1 \times 10^{-9}\) (cm\(^2\)/s) for Co\(^{2+}\);\(^{[49]}\) and \(t\) is the diffusion time, (s).

In the above equation, electrodynamic forces are ignored. Aggregation of nanoparticles allows hydrophobic clusters to permeate the lipid-bilayer either through ion channels (see (5)), or by increased permeation in the lipid bilayer after lipid peroxidation.\(^{[50]}\) The Co-TDM used as bactericidal agent created an acidic atmosphere, which serves as homogeneous catalyst to enhance the peroxidation of the lipid, causing cell death.

(2) Direct interaction: The phospholipid membrane structure can be stabilized by cations (Ca\(^{2+}\) and Mg\(^{2+}\))\(^{[51]}\) via ion binding between the anionic residues existing in the lipid head groups and cations in the nutrient media. The phosphate group (PO\(_4^{3-}\)) is generally considered to be the preferred cation binding site.\(^{[52]}\) With introduction of Co-TDM, direction interaction can occur between the cobalt ion (Co\(^{2+}\)) and anion in (such as PO\(_4^{3-}\)) at certain sugar residues; concurrently, the carboxylic (-COOH) group in the Co-TDM will bind with Ca\(^{2+}\) or Mg\(^{2+}\). Both interactions will lead to the formation of CO\(_3\)(PO\(_4\)), which result in pro-oxidative stress through generation of a reactive oxygen species (ROS, also see mechanism (3))\(^{[53]}\) on the cell membrane. ROS-induced stress and mechanical damage on the cell wall by the nanoparticles can then result in the weakening of the wall and lysis. The observed changes of membrane structure from TEM (Figure 4) and EELS (Figure 5) analyses are indicative of damage to the cytoplasmic membrane when bacterial populations were treated with bactericidal agent, Co-TDM.

(3) Generation of reaction-oxygen species: It has previously been demonstrated that Co\(^{2+}\) ions are capable of generating reaction-oxygen species (ROS).\(^{[54]}\) Metal-assisted radical
formation principally hydroxyl radicals (•OH) formation can cause modifications to deoxyribonucleic acid (DNA), leading to four distinct processes under the category of ROS-induced damage; namely (3a/b) unzipping of the macromolecule; (3c) enhanced lipid peroxidation and (3d) disruption of ion homeostasis. This •OH formation will also result in (3c) alterations to calcium (Ca²⁺) and thiol (SH) balance[53] and (3a) inhibition of DNA repair through cross-linking resulting in DNA unzipping and fragmentation (which was observed in our study).[54] In another study, it was demonstrated that cobalt (in conjunction with iron) had a stimulatory effect on lipid peroxidation in fatty acid micelles and phospholipid (phosphatidylethanolamine) liposomes, respectively,[55] noting that similar fatty acids are found in the lipopolysaccharides layer external to the peptidoglycan layer of Gram negative E. coli.[56] This stimulatory effect was not observed in our study indicating that inhibition/stimulation may be concentration dependent and above threshold are inhibitory, as was observed for membrane depolarization (also see §2.3.2 EELS Elemental Mapping of the Intact and Damaged Bacteria).

We further speculate that the Co-TDM motif would also be effective against Gram-positive microbes such as Staphylococcus aureus, since those microbes have lipoteichoic acid in their peptidoglycan layer,[58] which can serve as chelating agents. This chelating agent may serve as a mechanism to inhibit potential lipid peroxidation akin to how lipid peroxidation can be minimized using ethylenediaminetetraacetic acid (EDTA).[59] It was also shown that in the presence of cobalt, the inhibitory effects of EDTA against lipid peroxidation disappeared. It is plausible to speculate that any protective effects of lipoteichoic acid could be negated by Co-TDM, resulting in membrane damage through lipid peroxidation as one potential mechanism for inactivation for both Gram-negative/positive microbes.[60]

(5) Chelation Effects: Intriguingly, chelation of metals is one general mechanism whereby microbes exhibit tolerance to certain metals, which ordinarily would be toxic. In E. coli, for example the rcnA gene encodes for a membrane-bound polypeptide facilitation nickel/cobalt efflux,[61] resulting in competition between cobalt/iron for iron uptake at proteins, which utilize iron as a co-factor. One class are iron-sulfur proteins, which in E. coli contain at least three separate [Fe-S] cluster biosynthesis systems (genes names ISC, SUF enzymes named IscS, SufSE)[62] with cysteine supplying the atomic sulfur catalyzed by cysteine desulfurase in conjunction with certain structural proteins. These proteins provide a variety of ancillary functions such as cluster assembly/access to cluster precursors of [Fe-S] or iron chaperone or iron-carrier intermediates.[63] Co-TDM toxicity is related to decreased iron metabolism viz-a-via the [Fe-S] cluster assembly, which are chelated by scaffold proteins involved in iron-sulfur protein cluster biosynthesis, resulting in inactivation of related iron-sulfur enzymes (ferriochrome reductase, aconitate, and tRNA methylthio-transferase) leading to inhibition of certain critical proteins and toxicity.[64] Therefore, any metal nanoparticle system or chemical agents which can putatively interact with the ISC/SUF system and cause oxidation of the cluster proteins to the apoprotein form could be inhibitory. Recently, it was shown that hydrogen peroxide also disrupt Isc by oxidizing [Fe-S] clusters as assembled on/from the IscU scaffold, the exception being the class of microbes, which are able to switch over to another independent SUF pathway triggered by OxyR regulon are not H₂O₂-sensitive due to another enzyme (isopropylmalate isomerase) being able to repair the oxidative damage caused by H₂O₂ and also activate incipient Fe-S enzymes.[65] Hypothetically, therefore the Co-TDM would be inhibitory in the former case but not the latter, unless isopropylmalate isomerase were also to be oxidized.

(6) Membrane depolarization: For Gram-negative microbes, inactivation is through membrane depolarization induction resulting in transport of monovalent cations such as K⁺ across the cytoplasmic membrane of E. coli, but not divalent cations, which was observed in our study. The source of this depolarization was not determined in the current study, but has been postulated in the literature to occur between interactions with the cytoplasmic membrane.[66] This interaction between the inducer agent (e.g. nanoparticle) and membrane would result in the formation of ion channels, between the inner and outer cell membrane,[67] similar to channels formed by induction of E. coli with inducers such as colicins.[68]
IA, referred to as distilled water), was used where water-based solvents were necessary. All solvents were reagent or high performance liquid chromatography (HPLC) grade.

Single crystals with the formula \([\text{Co}_2(\text{H}_2\text{O})_3(\text{TDM})][\text{H}_2\text{O}]\) (abbreviated as Co-TDM) were prepared by mixing the ligand \(\text{H}_4\text{TDM} (0.05 \, \text{g}, 6.3 \times 10^{-5} \, \text{mol})\) with \(\text{Co(NO}_3)_2 \cdot 6\text{H}_2\text{O} (0.15 \, \text{g}, 5.2 \times 10^{-3} \, \text{mol})\). To adjust the acidity of solution, 20 drops of 2M \(\text{HNO}_3\) was added and \(\text{N,N-dimethylformamide (DMF, 18 mL)}\) was used as solvent. The solution was then sealed in a 20 mL vial and placed in an oven at 85 °C, where temperature was maintained for 24 h.

4.2. X-ray Crystallography

Single-crystal X-ray data for Co-TDM were collected using beamline 15-ID-B at the Advanced Photon Source, Argonne National Laboratory. A Bruker Smart Apex II diffractometer equipped with a low temperature device and synchronous X-ray energy (\(\lambda = 0.41328 \, \text{Å, silicon monochromated}\)) and a wide arrayed CCD (charge coupled device) detector were used to identify the crystalline structure in high resolution. Due to an insertion device in the beamline, an undulated narrow beam provided the highest theoretical sensitivity as opposed to the use of traditional single-crystal X-ray diffraction measurements. Adsorption corrections were applied using the SADABS routine. The structure was solved by direct methods and refined by full matrix least-squares on \(F^2\) with anisotropic displacement using the \(\text{SHELXTL}\) software package.\(^{[60]}\) Non-hydrogen atoms were refined with anisotropic displacement parameters during the final cycles. Hydrogen atoms connected to carbons were calculated in ideal positions with isotropic displacement parameters. The diffused electron densities resulting from these residual solvent molecules were removed from the dataset using the \(\text{SQUEEZE}\) routine of \textit{PLATON} and refined further using the data generated.\(^{[70]}\) The contents of the solvent region are not represented in the unit cell contents in the crystal data. Crystallographic data (excluding structure factors) for the structure(s) reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-835378. Copies of the data can be obtained free of charge from www.ccdc.cam.ac.uk/data_request/cif.

4.3. Electron Nanostructural Characterization

A FEI Tecnai G²-F20 transmission electron microscope (FEI Company, Hillsboro, OR) equipped with X-ray energy dispersive spectrometer (EDS) capabilities was employed to obtain nanostructure information and crystalline phase data about the colloid-derived nanoparticles. The instrument was operated in TEM mode to obtain the high resolution images of Ag-NPs, as well as scanning TEM (STEM) mode to obtain Z-contrast STEM images using a high-angle annular dark-field (HAADF) detector. The STEM mode utilizes a nanoscale electron beam to analyze the chemical composition of the intact and damaged bacterial cell structures. In both modes, the magnifications were calibrated using commercial cross-line grating replica and SiC lattice images.\(^{[71]}\) The spherical aberration coefficient and resolution were approximately 2.0 mm and 0.27 nm, respectively. A probe size of 1 nm was used for EDS and STEM, at 150 mm camera length for the HAADF detector to collect for Rutherford scattered electrons. Although a simplification, the collected image contrast is related to the atomic number (Z) squared, with elements with higher Z giving brighter contrast. The FEI Tecnai G²-F20 microscope was also equipped with a Gatan Imaging Filter (GIF) for electron energy loss spectroscopy (EELS). EELS was used to conduct the elemental mapping to determine the composition distribution. The elemental distribution mapping was made using a standard three window procedure, and the specific energy edges to image elements were listed as follows: 401 eV (N K), 284 eV (C K), 200 eV (Cl L₂,3), 132 eV (P L₂,3), 346 (Ca L₃), 294 eV (K L₂,3), 165 eV (S L₂,3), and 367 eV (Ag M₄,5).

The surface morphology and texture of the Co-TDM materials were examined using a JEOL 6701F field emission scanning electron microscope (JEOL Ltd., Plano, TX). The surface morphology and texture of the Co-TDM were also examined by a Quanta 600 FEG field emission scanning electron microscope (FEI Company, Hillsboro, OR). FESEM is capable of generating and collecting high-resolution and low-vacuum images. The instrument was equipped with a field emission gun and a Schottky emitter. The voltage was controlled at 5 or 15 kV and beam current at 100 nA. The chamber pressure and gun pressure were controlled to 3.5 × 10⁻⁵ Torr and 3.0 × 10⁻⁹ Torr for high resolution. A thin layer (4.0 nm) of gold (Au) metal was sputtered onto the sample surface to improve conductivity.

4.4. Bactericidal Activity Evaluation

The bactericidal activity assays were performed using \textit{Escherichia coli} (\textit{E. coli}; two commercially available strains, DH5alpha (Invitrogen, Grand Island, NY) and XL1-Blue, (Stratagene, Santa Clara, CA were used) as the model organism. \textit{E. coli} (5.0 mL) were cultured in Difco™ nutrient lysogeny broth (LB, Miller Luria-Bertani, Becton-Dickinson, Franklin Lakes, NJ) in an incubator shaker (Innova® 43, Incubator Shaker Series, New Brunswick Scientific, NJ) at 37 °C for 24 h. The cultured \textit{E. coli} were then diluted to 50 mL, into which the Co-TDM (0.50–0.75 mg) were introduced to evaluate the bactericidal activities. After the treatment, an aliquot (0.5 mL) was collected and tested using ultra-violet visible spectroscopy (Lambda 35 ultra-violet-visible (UV-Vis) spectrophotometer (PerkinElmer, Fremont, CA)) to identify the absorbance every hour in scan mode between 300-800 nm with a slit width of 5 nm, scan rate of 1 nm/min and fixed optical density mode at 600 nm. Control experiments were performed under identical conditions in the absence of the Co-TDM, disinfectant (e.g microbes in LB). In this study, we defined no growth of \textit{E. coli} cells as indicating full bactericidal activity and growth as indicating partial or no bactericidal activity. These were compared to values previously determined from studies with Ag, Ag₃O₂, and chemical disinfectant (phenol, sodium hypochlorite). Our previous experience in determining number of colon forming units (CFU) per milliliter for \textit{E. coli} using dilute samples (abs < 0.6) approximates to 1 OD unit corresponding to 10⁵ bacteria per ml, therefore OD₉₀₀ is an excellent implicit comparative technique in assessing
Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Bacterial viability, whereas plate dilution and counting of colonies is explicit and more accurate where the bacterial growth phase in not known or new strains are used for the first time.


