

# Uncertainties in the antibacterial mechanisms of graphene family materials

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1 *Opinion*

2 **Uncertainties in the antibacterial mechanisms of graphene**  
3 **family materials**

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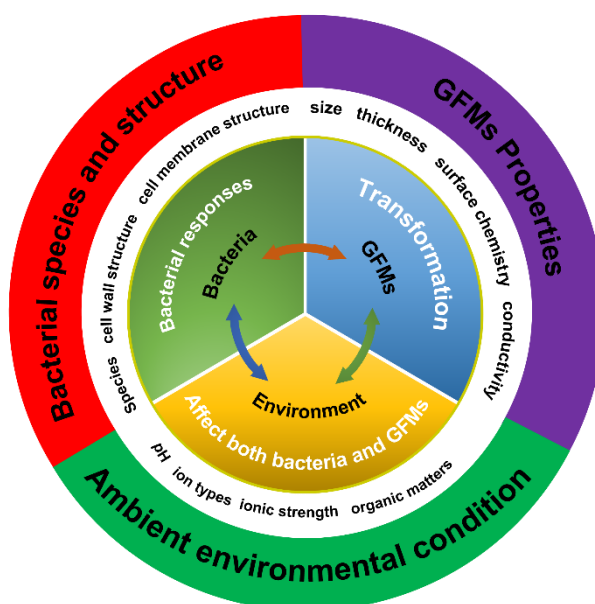
## ABSTRACT

The antibacterial activity of graphene family materials (GFMs) has been explored since 2010, 6 years after the discovery of graphene in 2004. It is proposed that the antibacterial activity is derived from both physical interaction and chemical reaction between GFMs and bacteria. However, whether the two mechanisms work synergistically or whether there are conditions under which one mechanism dominates remains uncertain. This opinion article highlights the uncertainties and controversies in the current understanding of antibacterial mechanisms of GFMs as well as deficiencies in methodologies and provides perspectives on future directions to move this field forward.

## KEYWORDS

Graphene family materials, bacteria, physical interaction, oxidative damage, computational modelling

## Graphic abstract



## 49 Introduction

50 Antimicrobial materials are an attractive approach for improving medical treatments, food packaging,  
51 wastewater treatment process, textiles, and dental care. Development of novel and efficient antibacterial  
52 agents is urgently required due to increasing bacterial resistance to existing antibiotics. Amongst the  
53 numerous nanomaterials that have been demonstrated to be bactericidal, graphene family materials (GFMs),  
54 especially graphene, graphene oxide (GO) and reduced graphene oxide (rGO), are undoubtedly attractive.  
55 Graphene is a 2D  $sp^2$ -hybridized carbon nanosheet composed of single-carbon atoms. GO is an oxidized form  
56 of graphene, while rGO is a form of GO with less oxygen content obtained by chemical, thermal and other  
57 reducing methods. The first report on the antibacterial performance of GFMs appeared in 2010, when Hu et  
58 al. produced GO paper with high antibacterial activity using a simple vacuum filtration method [1]. Since then,  
59 numerous studies have explored the antibacterial mechanism of GFMs [2] and developed GFM-based  
60 antibacterial materials including through modification with polymers [3], antibacterial metals [4] and  
61 nanomaterials [5].

62 The major reason for GFMs being attractive as antimicrobial materials is the hypothesis that there is much  
63 less chance of bacteria developing antimicrobial resistance (AMR) to GFMs because of their physical  
64 interaction mechanism [6, 7], compared with antibiotics which operate by interfering with RNA, protein or cell  
65 wall synthesis or DNA replication [8]. GFMs not only show AMR-independent antibacterial activity but also  
66 appear not to trigger long-term secondary resistance [6]. This unique feature allows GFMs to be used for  
67 various antimicrobial applications although the potential toxicity to environment and human health needs to  
68 be fully understood [9]. The physical mechanisms that have been proposed and most studied include side-on  
69 interactions with the sharp edges of graphene sheets that result in cutting [10], penetration and extraction of the  
70 lipid membrane [11] causing membrane damage and cell death, and wrapping [12] or trapping [13] of bacteria  
71 which starves the cells by reducing the ability to take up nutrients. Bridging effects, whereby GFMs act as a  
72 bridge to accelerate the movement of electrons between bacteria and the external environment, is also  
73 proposed as a mechanism that causes bacterial death [14]. When a physical mechanism dominates,  
74 development of antibacterial resistance is unlikely, as bacteria are not able to deactivate GFMs or evolve to  
75 modify the molecular target of the GFMs in bacteria. However, it has also been proposed that chemical  
76 reactions between GFMs and bacteria may also play a role in the antibacterial action [15], which increases the  
77 likelihood for development of antibacterial resistance. The chemical mechanism includes the self-generation  
78 of reactive radicals that kill bacteria or chemical oxidation of cellular components such as antioxidants (e.g.  
79 glutathione, GSH) which indirectly cause overload of reactive oxygen species (ROS) in the cell, causing cell  
80 death. Radical formation has been suggested to be less likely because the number of radicals generated by

81 GFMs *per se* is insufficient to produce such pronounced impacts [15, 16]. However, depletion of antioxidants  
82 has been proven to play a critical role in the antibacterial of GFMs. [14, 17-19].

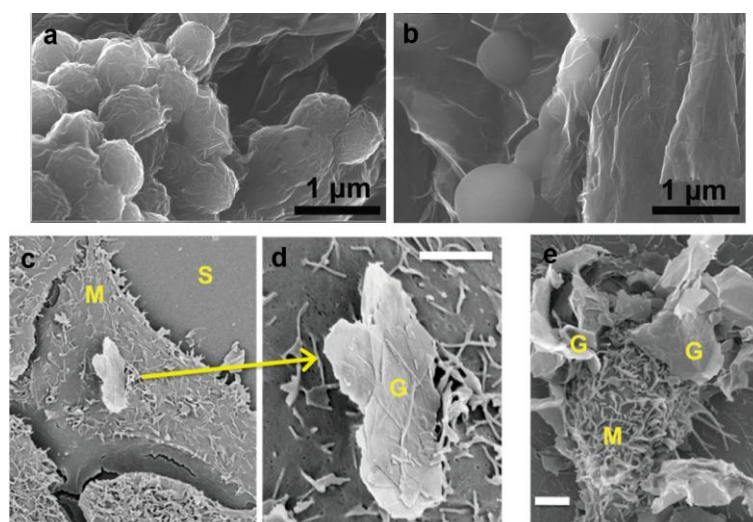
83 While we already know that these mechanisms play certain roles, it is unknown whether it is a simple  
84 combination of these or whether each mechanism is controlled by certain factors (e.g. medium, GFMs  
85 properties) and thus dominates under specific conditions. This fundamental question has been posed  
86 previously [2]; however, isolation of the individual physical and chemical mechanisms and untangling their  
87 relative contributions is challenging, so the question remains unanswered. Additionally, there are  
88 contradictions in the published literature regarding the effects of GFMs on bacterial growth, i.e. studies  
89 showing antibacterial effects, enhancement of growth, or insignificant effects. It is already known that the  
90 antibacterial activity are affected by many factors such as the experimental conditions, the bacterial species,  
91 and the physicochemical properties of the materials themselves. For example, changing the exposure medium  
92 from simple to nutrient-rich can reverse antibacterial effects and induce growth promotion [20]. Reducing the  
93 lateral size of the GO also enhances its antibacterial activity [21]. This opinion does not intend to discuss all  
94 the controversies associated with the antibacterial performance of GFMs. Rather, it will focus on some of the  
95 major uncertainties in the antibacterial mechanisms of GFMs, highlighting the drawbacks in the  
96 methodologies that lead to these uncertainties and provide direction on future studies to overcome them.

97

## 98 **1. Physical interaction mode: parallel or perpendicular**

99 The most well-accepted physical mechanism of antibacterial activity is the cutting of bacterial cell membranes  
100 by the lateral edge of GFMs. This concept was firstly proposed by Akhavan et al., who produced GO and rGO  
101 nanowalls with sharp edges that were nearly perpendicular to the stainless steel substrate and demonstrated  
102 excellent antibacterial activities [10]. However, later studies provide increasing evidence that parallel arrays  
103 of GO also kill bacteria, suggesting that perpendicular orientation is not a necessary condition for antibacterial  
104 activity of GFMs [22]. Note that in the aforementioned studies the GFMs are deposited onto substrates where  
105 their interaction mode is fixed, i.e. they are arrayed either perpendicular or parallel to the cells. In suspension,  
106 different interaction modes may occur simultaneously. However, many studies have reported wrapping or  
107 covering bacteria with GFMs, which represents a parallel interaction with the bacterial surface (**Fig. 1a**). The  
108 studies that observed wrapping modes exclusively used GO rather than rGO or pristine graphene. Edgewise  
109 contact with bacterial cells by rGO has been observed by scanning electron microscopy (SEM; **Fig. 1b**); however,  
110 whether such contact mode causes cutting or penetration of the bacterial cell membranes needs experimental  
111 evidence. The only study that visually identified the penetration of GFMs into cell membranes used pristine  
112 graphene and human cell lines (**Fig. 1c-1e**). Therefore, the dominant physical interaction mode of GFMs in  
113 suspension remains uncertain.

114 Fundamentally, the wrapping of GFMs on bacteria is driven by energy minimisation requirements,  
 115 whereby the more hydrophobic interiors of GFMs are shielded from water by promoting their stacking on the  
 116 hydrophobic cell wall of bacteria. Similarly, perpendicular penetration of GFMs into a cell membrane  
 117 maximises the hydrophobic interaction between the fatty acyl tails of lipids and the GFM surface. Since  
 118 wrapping mode is mainly demonstrated by GO, while rGO or pristine graphene predominantly interact  
 119 perpendicularly (**Fig. 1a and 1b**) [20], the interaction could be related to the surface oxygen content of GFMs.  
 120 Furthermore, the surface oxygen content is related to the mechanical properties of GFMs: GO is relatively soft  
 121 and flexible, readily able to wrap around bacteria [23], while rGO and graphene are rigid and free standing so  
 122 more likely to interact with cell membranes edge-wise [24]. If the mechanical properties of the material are  
 123 critical to the mode of interaction, we may deduce that other parameters such as lateral size and number of  
 124 layers may also play a role by indirectly affecting the mechanical strength. For example, small-sized GO  
 125 nanosheets may have more chance of contacting the bacterial cell membrane directly than larger sized ones  
 126 and are more likely to be free standing. Additionally, it is more energetically expensive for a larger graphene  
 127 sheet to align vertically with a cell membrane than smaller sheets, which are more likely to interact in this way  
 128 through Brownian motion. Increasing the number of layers increases the thickness of GO, thus making them  
 129 more rigid and increasing the chance of edge-wise contact while reducing their capacity for wrapping. Lastly,  
 130 the physical interaction of GFMs with gram-negative bacteria might be different from that with gram-positive  
 131 bacteria due to their distinct cell wall composition and structure. These hypotheses are still undemonstrated  
 132 and thus need further studies.

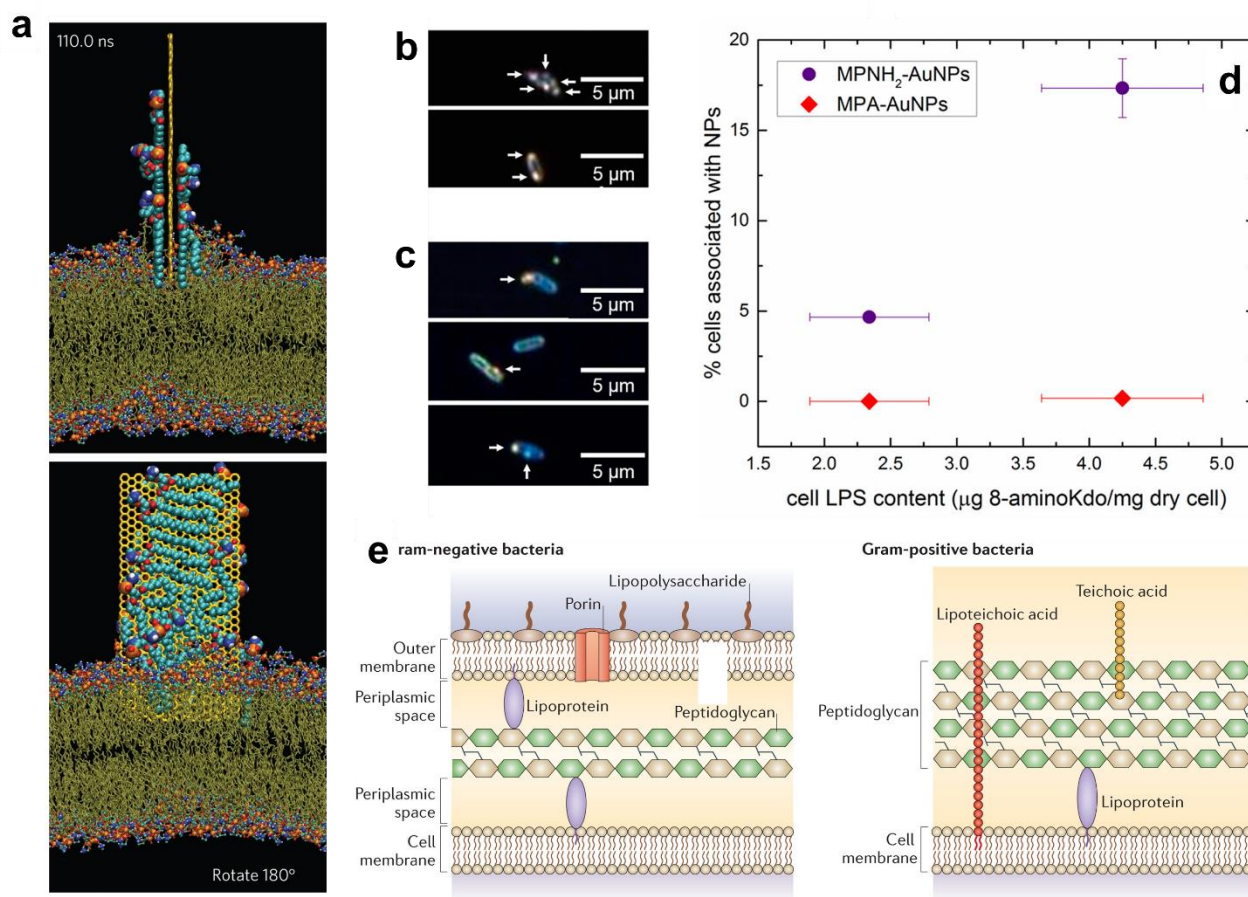


133  
 134 **Fig. 1. Physical interaction mode of GFMs with bacteria and cells.** **a, b**, SEM images of *S. aureus*, a gram-  
 135 positive round-shaped bacterium, after incubation with GO (**a**) and rGO (**b**) for 48 h. GO wrapped the bacteria  
 136 while rGO contact with the bacteria edge-wise. Adapted and printed with permission from [20], Copyright  
 137 2017, Elsevier. **c, d**, SEM images of human lung epithelia cells (A549) exposed to graphene for 24 h at low and  
 138 high magnification. Graphene penetrated the cell and stood freely. **e**, SEM image of murine macrophage

exposed to graphene for 5 h. Penetration of multiple graphene sheets into a single cell was observed. Scale bar in **d** and **e** indicate 2  $\mu\text{m}$ . Printed with permission from [25]. Copyright, PNAS.

## 2. Computational modelling needs to consider the bacterial structure

Computational modelling has been increasingly useful for obtaining mechanistic understanding of the interaction of nanomaterials including GFMs with biological systems at the molecular level. For example, the mechanisms of physical puncture and extraction of lipid membrane components have been proposed based on molecular dynamic (MD) simulations [11]. The usefulness of the computational modelling depends on the construction of the initial model and choice of parameters. Because of their large size, cells are usually represented by a simplified planar lipid membrane. Most studies to date have used a single phospholipid (POPE) or a combination of two lipids (POPE and POPC) to simulate the lipid membrane (**Fig. 2a**). However, the bacterial cell membrane also contains other components such as proteins, cholesterol and carbohydrates, which have been recently suggested to play important roles in the interaction of nanomaterials with bacterial cell membranes [26]. For example, positively charged gold nanoparticles tend to interact with gram-negative bacteria more than negatively charged gold particles mainly because of the interaction of lipopolysaccharides (LPS) (**Fig. 2b-2d**) [26]. Another key issue is that current models do not consider bacterial cell structure. The bacterial cell membrane is a complex multi-layered structure that protects them from hostile environments. The plasma membrane of both gram-positive and gram-negative bacteria is surrounded by a thin peptidoglycan cell wall (**Fig. 2e**). The former has a thicker cell wall, but while the latter has a thinner cell wall it is surrounded by an outer membrane containing LPS. It remains unclear whether GFMs can penetrate the peptidoglycan cell wall and enter the inner plasma membrane. Thus, we suggest that computational modelling might need to consider the cell wall and other membrane components in future studies. This could be combined with experimental studies to understand the role of a specific biomolecule component. Moreover, the biocorona that forms on the GFMs surface immediately after contact with a culture medium will also affect these interactions and should be considered in modelling, although it will increase the cost and time substantially.



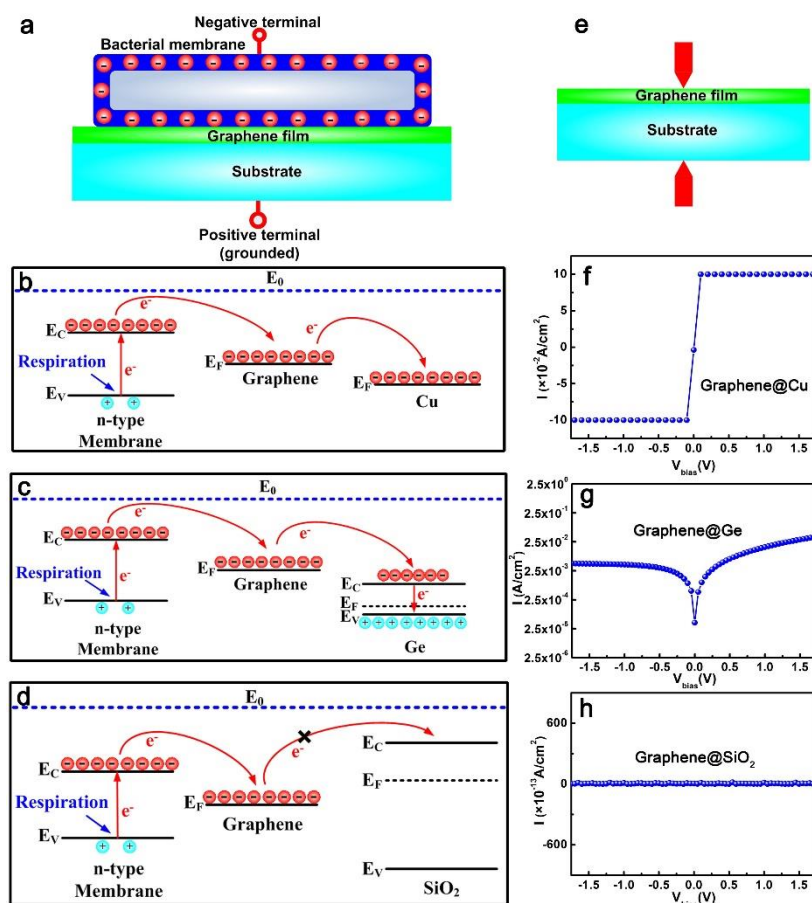
**Fig. 2. MD simulation and role of bacterial cell structure on interactions.** **a**, a representative trajectory of a fully restrained graphene nanosheet docked at the surface of the POPE lipid membrane. The simulation time is 110 ns and the lower snapshot is obtained by rotating the upper snapshot anticlockwise by 180 degrees. Printed with permission from [11], Copyright 2013, Spring Nature Group. **b**, **c**, **d**, Gold nanoparticle (AuNP) association with bacterial cells is directly observable and depends on the cell LPS content. The AuNP was functionalized with either 3-mercaptopropionic acid (MPA) or 3-mercaptopropyl amine (MPNH<sub>2</sub>). (b) Cells isolated (sorted) from the total cell population after exposure to MPNH<sub>2</sub>-AuNPs. (c) Unsorted cells after exposure to MPNH<sub>2</sub>-AuNPs. In panels **b** and **c** the arrows point to AuNPs associated with the cells as confirmed by hyperspectral imaging. (d) Association of MPA- or MPNH<sub>2</sub>-AuNPs with the marine bacteria *Shewanella* cells with varying LPS content (indicated by 8-amino-2-keto-3-deoxy-D-manno-octonate (8-aminoKdo) content of lyophilized cells) quantified by flow cytometry. Error bars (representing one standard deviation, n = 3) are smaller than the symbol in some cases. Printed with permission from [26], Copyright 2015, American Chemical Society. **e**, Schematic illustration of gram-positive and gram-negative bacterial structures. Printed with permission from [27]. Copyright 2015, Spring Nature Group.

### 3. Underestimated role of electric conductivity of GFMs



182 While physical puncturing, nutrient deficiency as a result of wrapping, and oxidative stress are widely accepted  
183 mechanisms of GFM- induced antibacterial activity, electric conductivity is a key property of GFMs that has  
184 been overlooked and less studied in terms of its role in the antimicrobial activity of GFMs. In eukaryotic cells,  
185 electron transport (ET) occurs in the mitochondria but in prokaryotes, which lack mitochondria, it operates in  
186 the plasma membrane, i.e. the single membrane in gram-positive bacteria or the outer membrane in gram-  
187 negative bacteria [28]. The ET is central to the production of the energy needed to support bacterial growth.  
188 When the ET in bacteria is disrupted, cell death occurs. In fact, enhancing ET has become increasingly attractive  
189 as an effective strategy for electrochemical antibacterial approaches [29]. The typical way to achieve this is  
190 by immobilizing electron acceptors (e.g. graphene or other nanomaterials) onto a metal or semiconductive  
191 substrate so that electrons are transferred to the nanomaterial-metal system from the bacterial membrane,  
192 causing membrane damage. Several studies have shown that immobilization of GFMs on conductive  
193 substrates (e.g. Cu, Zn, Ni) can significantly enhance their antibacterial activity [30, 31]. The ET mechanism is  
194 further supported by the fact that graphene and GO show little or no bactericidal effects on insulating  
195 substrates such as SiO<sub>2</sub> film [31] or glass [30]. This can be explained by the different band structures of  
196 conductors, semiconductors and insulators (**Fig. 3**). The respiratory protein in bacterial cell membranes, which  
197 is responsible for oxygen transport, storage and delivery, behaves as a semi-conductor with a bandgap of 2.6  
198 eV~ 3.1 eV. Contact between the cell membrane and a (semi) conductor will lead to the alignment of the  
199 Schottky barrier and Fermi level, which facilitates electron transfer from the membrane to graphene [31].

200 Note that these studies are all performed on a conductive substrate, which acts to enhance electron  
201 transfer so physical puncturing or wrapping of the bacterial cells is unlikely to happen. However, in suspension,  
202 e.g. in wastewater, direct evidence for the ET mechanism is lacking and other physical mechanisms may occur  
203 simultaneously. A study by Chong et al. reported that sunlight exposure can increase the antibacterial activity  
204 of GO which they attributed to light-induced electron-pair holes on GO enhancing the ET from antioxidants  
205 (e.g. GSH) [16]. Another study reports that nitrogen doping of GO eliminates their antibacterial activity  
206 because nitrogen has one more electron than carbon and thus changes GO from an acceptor to an electron  
207 donor, thus preventing energy transfer in the bacteria and reducing the antibacterial activity of GO [32]. While  
208 a few studies indicate that the electric conductivity of GFMs may play an important role in their antibacterial  
209 activity, in many studies GO shows higher antibacterial performance than graphene or rGO despite having  
210 lower electric conductivity. Thus, in suspension, multiple factors might act simultaneously and this needs to  
211 be studied and explored under specific conditions.



**Fig. 3. Role of electric conductivity of GFMs in their antibacterial activity.** **a** Schematic illustration of an electrochemical antibacterial device utilising GFMs. **b-d**, Energy band diagrams of graphene-on-substrate junctions on a conductor such as Cu (**b**), a semiconductor Ge (**c**) and an insulator SiO<sub>2</sub> (**d**) substrates. **e – h**, Schematic illustration for the electrical measurements (**e**) to obtain the current–voltage (**I–V**) characteristics of Graphene@Cu (**f**), Graphene@Ge (**g**) and Graphene@SiO<sub>2</sub> (**h**) contacts at room temperature, respectively, indicating three different types of contact of graphene films with the underlying substrates. Printed with permission from [31]. Copyright 2014, Springer Nature Group.

#### 4. Improved methodologies to study chemical mechanisms of action in bacteria are needed

The primary chemical mechanism for GFMs-induced bacterial death is believed to be mediated by oxidative stress. It can be caused either by reactive oxygen species (ROS) directly generated by GFMs or *via* the depletion of antioxidants which are responsible for capturing excessive ROS in order to maintain a balance in the bacterial cells. Although the mechanisms of ROS generation are still controversial, it is increasingly evident that the number of ROS generated directly by GFMs is minimal, contributing little to the antibacterial activity

[15, 16]. Thus, the oxidation of antioxidants such as GSH seems to be the key chemical mechanism. Indeed, *in vitro* GSH oxidation has been used as a classic method to demonstrate and compare the capacity of GFMs to oxidize the cellular antioxidants and to quantify the subsequent antibacterial effect [14, 33].

The question raised here however, is whether this *in vitro* test represents a realistic scenario in bacteria. Firstly, the bacterial species should be considered as a key factor because GSH is widely found in eukaryotes and gram-negative bacteria but is hardly present in gram-positive bacteria [34, 35]. This means that GSH oxidation assay is not ideal when evaluating antibacterial activity towards gram-positive bacteria.

Secondly, *in vitro* GSH oxidation assays are performed in liquid phase and the reaction is based on direct contact of the GFMs with GSH. In cells, however, GSH is only produced in the cytosol and efflux to extracellular regions is low. Besides, as there is no direct evidence to date showing that GFMs can enter bacterial cells, the possibility of direct interaction between GFMs and GSH is very low. Although computational modelling studies suggest that graphene can penetrate cell membranes, experimental evidence of the entry of GFMs into the cytosol has only been reported in mammalian cells through endocytosis [36]. However, endocytosis is not known to occur in bacteria [37]. Unless the bacterial cell wall and membrane are broken, GFMs are unlikely to enter the bacterial cytosol and reach GSH. The cell wall is unlikely to be broken by GFMs under pure physical forces, unless chemical interactions between GFMs and bacterial cells occur to rupture the cell wall and allow entry of GFMs into the cells. Therefore, more experimental evidence is needed to demonstrate the direct oxidation of GSH by GFMs in bacteria. This may need *in situ* techniques such as labelling to observe GFMs inside bacteria at a subcellular level. Alternatively, GSH oxidation may occur indirectly, e.g. *via* an electric conductive bridging effect. GFMs may act as a conductive bridge over the insulating cell membrane, accepting electrons transferred from GSH [16].

Lastly, the GSH assay is usually performed in buffer without considering the medium in which the bacteria are cultured. *In vitro* GSH oxidation relies on direct contact with GFMs. Upon contact with the medium, the physiochemical properties of GFMs may change immediately due to the adsorption of ions or biomolecules onto the basal surface (formation of a biocorona) [38]. Thus, to interpret the results thus to determine link with antibacterial activity, pre-incubation of GFMs in relevant culture media might be necessary in future studies.

## 5. Summary and outlook

Despite the growing body of literature on the antimicrobial activity of GFMs, there are still several uncertainties in the mechanism of this behaviour / effect. To explore these mechanisms, several fundamental questions need to be answered in the near future, including:

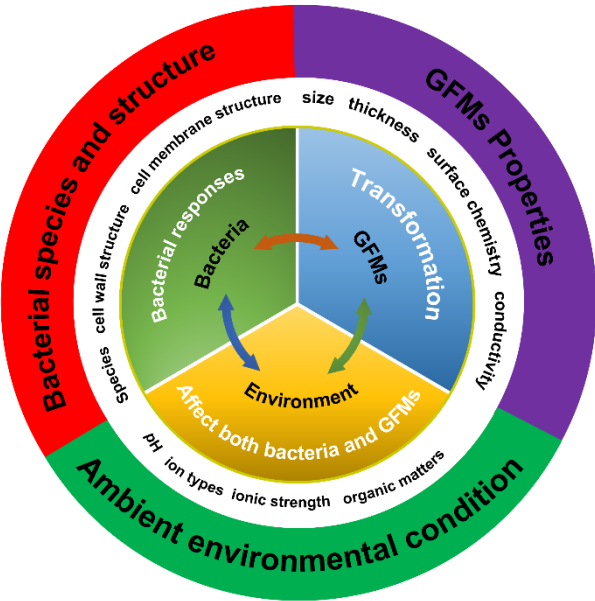
260 1) *What are the main factors driving the interaction mode?* It has been demonstrated that the surface oxygen  
261 content of GFMs can switch the interaction mode from parallel to perpendicular. Whether other parameters  
262 such as sheet size or thickness can affect the interaction mode needs to be understood. The interconnection  
263 of these parameters and the combined effects of the GFM physicochemical properties as well as the impact  
264 of the medium composition on these, should be explored and understood.

265 2) *Can GFMs cut cell membranes and enter bacteria?* Computational simulations have provided vital  
266 mechanistic information that GFMs may cut the cell membrane and extract the lipid membrane components.  
267 However, a limitation is that these simulations are based on a simplified lipid membrane model. More  
268 experimental and computational research is required to demonstrate the role of other membrane or cell wall  
269 structural components in the interaction of GFMs with bacteria. While it is difficult to incorporate all the cell  
270 components into a single simulation system because of the significant computational cost and time, which will  
271 remain a challenge in the foreseeable future, this can be studied separately and interpreted together with  
272 experimental data. Experimental evidence is also needed as to whether GFMs can cut through cell walls and  
273 enter bacteria when in suspension. Sophisticated techniques such as isotope labelling of GFMs and/or imaging  
274 of their location in a single bacterial cell may be needed. Besides, machine learning may make it feasible to  
275 obtain critical information from data accumulated over the last decade, when combined with molecular  
276 dynamics and coarse-grained simulation.

277 3) *Is there an alternative method for examining the oxidative potential in vivo?* As discussed above, the *in vitro*  
278 GSH oxidation assay is not suitable for most gram-positive bacteria. Can other intracellular antioxidants be  
279 used as alternatives for GSH?

280 The complexity of antibacterial activity and its mechanisms are not only the result of the  
281 physicochemical properties of GFMs but also different bacterial species and the dynamics of the environment  
282 in which they are present. Although antibacterial properties could be driven by multiple factors simultaneously,  
283 increasing evidence suggests that they are dominated by certain factors (e.g., size, SOC or culture medium  
284 composition) in specific scenarios, which need to be elucidated. Therefore, systematic studies are required in  
285 the future. More importantly, these factors are interconnected so an integrated view of their roles is needed  
286 (**Fig. 4**). The biological and chemical activities of GFMs are determined by their physicochemical properties  
287 including lateral size/surface area, thickness (number of layers), surface chemistry and electrical conductivity.  
288 However, the original identity of GFMs can change when they enter a biological environment, e.g. through  
289 agglomeration or formation of a biocorona, which determines the subsequent biological activity of the GFMs.  
290 The physical and chemical mechanisms involved in GFM-bacterial interactions, in essence, may become GFM-  
291 biocorona-bacterial interactions. Thus, bacterial responses to GFM exposure vary in different environments.

Bacterial species are also critical given their structural differences and that they may excrete different extracellular components, which affect their interactions with GFMs.



**Fig. 4.** Illustration of the interconnection of the properties of GFMs, bacteria and the ambient environment. GFMs can both inhibit or enhance bacterial growth, and which effect they induce is affected or even reversed by changing the growth conditions. Bacterial growth depends on the ambient environment. Bacteria secrete biomolecules and their respiratory activity can alter the properties of GFMs, causing biotransformation, which subsequently affects the antibacterial effect of the materials. Untangling and exploiting these interconnected processes is essential to ensure the safe use of GFMs.

These critical factors including physical / chemical properties and experimental conditions, however, are not always fully reported. For example, SOC might be a key factor determining whether GFMs interact with bacteria perpendicularly or edge-wise (**Fig. 1a and 1b**); however, based on our survey of the literature regarding the antibacterial effects of pristine GMs since 2010, only 23 out of the 72 studies (32%) reported SOC. Lack of reporting of SOC renders the results from these studies difficult to compare. To enable a comparison between different studies and address the uncertainties in the mechanisms of action of GFMs, we suggest a checklist of questions for performing antibacterial tests with GFMs. The impact of each aspect of the material, medium, and bacterial species is analyzed. We recommend that this checklist be used as a standard for future studies exploring the antimicrobial properties of GFMs.

**Table 1.** Checklist for reporting GFMs antibacterial studies to enable comparison between different studies.

Reporting standard for GFMs antibacterial study	Techniques	Impact
-------------------------------------------------	------------	--------

Are the GFMs fully characterized?	Method of synthesis	N/A	Synthetic method may lead to different physicochemical properties of GFMs and thus should be described
	Impurities/ doping or composites	XPS, ICP-MS	Impurities such as sulphur <i>per se</i> can induce antibacterial effect, or doping such as nitrogen affects the antibacterial effects, and thus should be quantified, including potentially their release kinetics in the exposure medium
	Lateral size or surface area	TEM, SEM, AFM	Larger-sized GFMs have higher antibacterial activity while the effects can be affected by other factors simultaneously
	Thickness or number of layers	AFM	Thickness affects the edgewise contact of GFMs with the bacteria, which may reduce the “cutting” effect
	Surface oxygen content or C/O ratio	XPS	Surface oxygen content affects the properties of GFMs (e.g. rigidity, electric conductivity, hydrophilicity) thus the interaction of GFM with bacteria
	Surface charge	DLS / zeta potential	Surface charge affects the interaction of GFMs with the bacterial surface
Are bacterial species accurately reported?	Bacterial species and strains	N/A	Different species respond differentially to the same GFMs
	Growth stage	N/A	At different stages in their growth curve the bacteria respond differentially to GFMs
Are culture media compositions reported clearly?	Name and compositions of the media	N/A	Different culture media affect the agglomeration state of GFMs. Rich media change the surface by forming a biocorona on the GFMs surface
Are exposure method and duration clearly described?	Exposure method and duration	N/A	Results can be different due to the variation in the test method (suspension assay, biofilm assay, colony counting test) and duration (3h, 24h or more)
Are relevant parameters clearly reported for computational modelling?	GFMs properties, model cell membrane composition, medium composition, interaction duration etc	Computational simulation	One parameter (e.g. size, surface oxygen content of GFMs, or medium pH, organic matter content) may change the interaction of GFMs with the cell membrane

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