Confined, Oriented, and Electrically Anisotropic Graphene Wrinkles on Bacteria

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Supporting Information

ABSTRACT: Curvature-induced dipole moment and orbital rehybridization in graphene wrinkles modify its electrical properties and induces transport anisotropy. Current wrinkling processes are based on contraction of the entire substrate and do not produce confined or directed wrinkles. Here we show that selective desiccation of a bacterium under impermeable and flexible graphene via a flip-valve operation produces axially aligned graphene wrinkles of wavelength 32.4–34.3 nm, consistent with modified Föppl–von Kármán mechanics (confinement ~0.7 × 4 μm²).

Further, an electrophoretically oriented bacterial device with confined wrinkles aligned with van der Pauw electrodes was fabricated and exhibited an anisotropic transport barrier (ΔE = 1.69 meV). Theoretical models were developed to describe the wrinkle formation mechanism. The results obtained show bio-induced production of confined, well-oriented, and electrically anisotropic graphene wrinkles, which can be applied in electronics, bioelectromechanics, and strain patterning.

KEYWORDS: graphene, bacteria, anisotropy, wrinkles, flip-valve, bioelectronics

Ultrathin, flexible, two-dimensional nanomaterial (2DN) sheets, such as graphene, boron nitride, and transition metal dichalcogenides (MoS₂, WS₂, etc.), can form wrinkles,3 crumples,4 and folds.4 These corrugations in free graphene result in local strain distribution and curvature-induced rehybridization of the p-cloud, which modify (a) the electronic structure,6,7 local charge distribution,8 dipole moment,9 and optical properties3 of graphene and (b) its local chemical potential due to the formation of electron–hole puddles.10 These modified electrical properties can then be applied toward electronics, self-assembly of complex structures, nanoelectromechanics, and bioelectronics. However, confined and directed wrinkle formation in 2DNs is still a challenge. Here, this challenge was addressed by employing a bacterial cell as a contractible scaffold that can be deposited at specific locations via electrophoresis and where graphene deposited atop can form wrinkles.

Integrating the properties of 2DNs with the functionalities of interfaced biological components has produced advanced bionanotechnologies in sensing,11,12 bioactuated devices,13,14 and biogated field-effect-transistors (FETs).15,16 Most of these efforts have focused on electrochemical devices on supported (nominally flat) graphene. Since graphene’s electrical properties are sensitive to its morphology (as mentioned above),5,7 mechanical actuation by the cell of the interfaced graphene can produce wrinkled graphene with modified properties. We employ rod-shaped Bacillus subtilis cells for this work for their following attributes: (i) nominally rod-shaped (orientable), (ii) size of 1 × 5 μm² (can produce confined wrinkles), (iii) long and connected chain forming bacteria (for longer wrinkles), and (iv) highly volatile intracellular content (~75–80% water) (shrinkable: cell contracts with water reduction). Our previous work showed that graphene atop a cell retains its aqueous content under high vacuum enabling wet microscopy.17 Here, we designed a graphene flip-valve via interaction of monolayer graphene with a biological cell (B. subtilis), which permanently removes the bacterium’s aqueous content under vacuum (Figure 1a). This mechanism introduces compressive strain to form axially aligned wrinkles on graphene. As will be shown

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Graphene is impermeable and strong, this crack creates a graphene pathway for volatile cellular content to escape. However, the minimal axial shrinkage and the creation of longitudinal (energetically favored) radial shrinkage of the bacterium closing (sealing graphene on SiO2) of the graphene where the pressure difference drives the water out, and to the aperture operation, radial (transverse) wrinkles on graphene (about 500 nm of length and 70 nm of width) are produced. These are attributed to its Poisson’s ratio (ν = 0.165 = −εy/εx). Here, graphene’s interaction with the additional perimeter of the protruded curved bacterium causes graphene to undergo tensile strain (mostly in the radial direction). This leads to graphenic compression in the axial/longitudinal direction (due to the Poisson’s ratio), forming the radial wrinkles (Figure 1b).

The graphene wrinkles formed are longitudinal and confined atop the bacterial cells (and sometimes connected between neighboring cells) as observed under field emission scanning electron microscopy (FESEM) as shown in Figure 2. Since the FESEM micrograph contrast corresponds to electron scattering due to surface curvature (as on wrinkles) and electron density, the contrast pattern (inset of Figure 2) provides the number of wrinkles (n) on each transverse bacterium with an average wavelength, λ (distance between two wrinkles), of 32.4 ± 3 nm (see Supporting Information Section 2 for calculation details).

The wrinkle wavelength is independent of the diameter of the bacteria, since (a) the wrinkle wavelength is a consequence of the balance of graphene’s bending energy and the bacterium’s contraction energy, both of which are relatively unaffected by the bacterial diameter, and (b) the bacterial curvature is significantly smaller than the wrinkle curvature (Dbacteria/λ wrinkle = 20–35) to have a significant geometric effect on the wrinkle wavelength (as shown in Supporting Figure S3). The angular image analysis depicts that the texture direction (TD) of the selected wrinkled area is 89.7 ± 0.6° (close to 90°) (Figure 2b), implying longitudinal directionality of wrinkles. Further, a surface with a texture aspect ratio (TAR) of 0.125 (<0.3) (Figure 2c) for the wrinkled graphene indicates strong spatial anisotropy.

The atomic force microscope (AFM) scans of graphene interfaced with bacteria after vacuum and heat treatment as shown in Figure 2d and e are consistent (wavelength = 34.3 ± 2 nm) with FESEM image analysis in Figure 2a.
AFM results shown in Figure S4 and wavelength calculation details outlined in Supporting Information section 1 and Figures S5 and S6. The height of the wrinkles varies from 7 to 10 nm. Unlike FESEM, which operates under high vacuum, AFM allows inspection under atmospheric pressure (Figure 2).

The relative strain on graphene at different stages of the graphene–bacteria interaction was investigated via Raman shift measurements of the G and 2D peak positions as well as their shapes.\(^{24}\) The elongation and weakening of the C–C bonds lead to a Raman red-shift (lowering of the vibrational frequency). The peak’s full width at half-maximum (FWHM) increases upon application of tensile stress.\(^{24}\) The Raman mapping of graphene on the bacterial surface before and after vacuum treatment is displayed in Figure 2h and i, respectively.

Both the G and 2D peak positions of graphene on the bacterium’s surface (Figure 2j and k and Figure S8) are red-shifted compared to graphene on a flat substrate (darker on the bacterium than on SiO\(_2\)/Si, indicating the existence of tensile strain induced by bacterial curvature (FESEM and AFM).\(^{24}\) The strain also increases the FWHM of the G peak from 15 cm\(^{-1}\) to 33 cm\(^{-1}\) (more than 2-fold). After vacuum/heat annealing and wrinkle formation, graphene on bacteria exhibits a blue-shift in the G peak with respect to graphene on SiO\(_2\)/Si (brighter on the bacterium than on SiO\(_2\)/Si). This Raman blue-shift can be attributed to the release of tensile strain or addition of compressive strain due to the formation of wrinkles. Further, the entire graphene region exhibits a blue-shift (~15 cm\(^{-1}\) increases in G and 2D peak positions) attributed to desorption of O\(_2\) and other adsorbents.\(^{26}\) The 10 cm\(^{-1}\) red-shift of the 2D peaks implies a strain of 0.37% for graphene on the bacterium (see the Supporting Information, Section 4). Studies with other cell types and strains are important; however, these are beyond the scope of the current work.
In order to study the effect of graphitic wrinkles' orientation on the carrier transport, we fabricated a “plus-shaped” wrinkled-graphene-on-bacteria device (van der Pauw structure) with bacteria placed parallel and perpendicular to a pair of respective electrodes (Figure 3a). The FESEM and AFM micrographs confirm that the graphene wrinkles are oriented in the longitudinal direction. The Raman scan images clearly show that graphene stays atop Au/Cr electrodes after wrinkle formation, and there is no separation of graphene from the electrodes. The transverse length (or width) of graphene on bacteria is about 1 μm and on SiO2 is 9 μm with excess graphene on the Au/Cr electrodes. Therefore, if there is any contraction causing graphene to slide, there will be excess on the electrode.

Current–voltage (I–V) characteristics under vacuum exhibited lower conductivity in the transverse direction (T-direction) than in the longitudinal direction (L-direction) (at all applied gate voltages). Out of the 10 devices tested, the doping levels were varied and the Dirac point (with p-doping) was observed for only two devices (Figure 3d). At respective Dirac points (zero net doping, n = p), the conductivity in the L-direction was higher than that in the T-direction. Further, the Dirac point for L-transport was ~8 V higher than that for T-transport, or in other words, L-transport had more holes (<1.46 × 10^{11} holes/μm²) (as carrier concentration n = 2CSO₂ΔV/e). This represents the difference in the participating doped charges from bacteria along the two conduction pathways: The least resistive path in the L-direction has more bacteria (thus more doping) than in the T-direction. This also explains the sharper Dirac point in the L-direction (Figure 3d). Clearly, the bacteria significantly p-dopes the overlaying graphene sheet. It is important to note that the net gate capacitance will include the capacitance from SiO2 and the bacterial dielectric (C_{gate,net}⁻¹ = C_{SiO₂}⁻¹ + C_{bacterium}⁻¹). The other eight devices exhibited reduction in current with increase in gate voltage from −85 to +85 V (Figure 3c) for temperatures ranging from 10 to 300 K (beyond 85 V, dielectric breakdown started). The variation in the overall doping is attributed to bacteria of different ages having different surface potentials.28

To determine the transport activation barrier, temperature studies were conducted at reduced doping levels at the maximum gate voltage (85 V) (Figure 3b). Two conduction regimes were identified: (i) high temperature (120 to 300 K) and (ii) low temperature (10 to 120 K). The activation energy (\textit{I} \propto \exp(-E_a/kT)) for the high-temperature regime in the T-direction was 1.69 ± 0.01 meV higher than that in the L-direction. The Schottky barriers at the electrode–graphene junctions are expected to be similar for L- and T-transport, indicating a slightly increased barrier for transverse transport. Note that the barrier must not be affected by the carrier concentration. Therefore, the anisotropic wrinkles lead to suppressed carrier mobility in the T-direction.1 This is speculatively attributed to the additional barrier due to local charge concentration distribution (and potential distribution) following the pattern of the longitudinal wrinkles (for example, electron–hole puddles may be patterned via the wrinkles).
Further, since for folds the T-conductivity is expected to be higher, it confirms that this is a wrinkle-intensive device. Also, at reduced temperature, the thermal expansion of graphene is expected (−ve thermal expansion), which might lead to relaxation of wrinkles. This is consistent with the significantly reduced activation energy for the low-temperature regime. Further work is required to obtain measurements by using a different cell (for example, noncharged Gram-negative cell with no peptidoglycan membrane) to study the modification in the band structure of wrinkled graphene.

Mechanisms of Graphene–Bacteria Interaction. To understand the mechanism on free graphene interfaced with a biological cell, coarse-grained molecular dynamics (CGMD) simulations were performed. Here, the graphene sheet is pulled by the curved bacterium, creating transverse wrinkles (Figure 1b) (see Supporting Movie S2). Clearly, the presence of radial wrinkles implies pretension in the graphene membrane on the bacterium. We also studied in-plane strain along the cross-section profile of graphene/bacterium contact as 0.42 ± 0.01% in the CGMD simulations, which is in good agreement with strain measured via Raman spectroscopy. Post-vacuum/heat treatment, the size of the bacterium model was set to shrink by 40%, consistent with the experimental conditions. The simulation incorporated the structural relaxation after shrinking, which results in the generation of wrinkles on graphene on the bacterial surface. The process starts from nucleation of wrinkles with small wavelengths and continues by merging of a converged configuration with stabilized wavelength that is defined by the amplitude of shrinking as well as the elasticity of the bacteria and graphene (as shown in Figure S10 and Supporting Movie S3). The stabilized wavelength of wrinkles predicted using the physical parameters for the system is about 34 nm, which is also consistent with the measured average wrinkle wavelength (simulation details and parameters are provided in the Supporting Information).

The crack formation on graphene under heat and vacuum treatment is attributed to two mechanisms: (a) opposite polarity of thermal expansion coefficients between the graphene (−ve thermal expansion coefficient) and bacteria (+ve thermal expansion coefficient) and (b) radially induced tensile stress due to the applied pressure from volatile bacterial content (estimated to be ~100 bar; see Supporting Information). The region where graphene transitions from the bacterium to the SiO2/Si substrate experiences a high differential strain due to a change in curvature (bacterium–SiO2/Si boundary) and high tensile stress due to the large pressure difference across
graphene (pressurized volatile cellular matter to vacuum). The tensile strain causes longitudinal crack nucleation at the inflection points near the substrate, as shown by extended finite element method (XFEM) simulations: the crack is formed perpendicular to the stress direction (Figure 4e and Supporting Movie S4). The width of the cracks (speculatively zigzag edged) was mostly independent of the bacterial size at about 200 nm. In some cases, the graphene/bacteria system under vacuum did not have to be heated to form cracks (attributed to defects on graphene), which were always on the bacterium/substrate interface. It should be noted that cracks formed only on graphene-wrapped immobile bacteria on the substrate but not on graphene-wrapped free-standing bacteria, as shown in Figure S11.

We also performed atomistic molecular dynamics simulations to better elucidate the interface and adhesion between graphene and the bacterial cell wall for a single-wrinkle peak (17 nm wide, similar to the experimental results, shown in Figure 4g and Figure S11) on bacteria covered with graphene. The simulated system consists of graphene interfaced with the bacterial cell wall: peptidoglycan layer (thickness 25–30 Å, 100 × 600 Å²), lipid bilayer (80 × 550 Å²), cytosol (protein, 0.18 M ions and hydrating water) and water layer, from top to bottom, respectively. The simulation detail is described in the Supporting Information. The simulation clearly shows that graphene stays strongly adhered to the bacterium cell wall, implying a high adhesion energy (calculated 218 mJ/m² in graphene and the bacterial cell wall for a single-wrinkle peak) acting on graphene. Further, small folds within the wrinkle were also observed (however, not resolved by experiments).

The morphology correlates with a Young’s modulus of the substrates. Micro- and macroscopic wrinkles are ubiquitous in clothes, leaves, animal skins, dried fruits (raisins, dates (Figure 1d)) and other surfaces. Nanoscale wavelengths require ultrathin skin as per the Föppl–von Kármán relationship:52

$$\lambda = 2\pi \sqrt{\frac{E_{2DN}}{3S E_S}}^{1/3}$$

where \(\lambda\) is the wrinkle wavelength, \(t\) is the thickness of the 2DNs, \(E_{2DN}\) is the plane-strain modulus of the 2DNs film, and \(E_S\) is the in-plane strain modulus of the substrate. \(E = E/(1 - \nu^2)\), where \(E\) is Young’s modulus and \(\nu\) is Poisson’s ratio. Equation 1 assumes a strong adhesion between 2DNs and the substrate with no slip between the two layers. Since it is the thinnest material, graphene’s wrinkle wavelengths are expected to be small. Further, \(\lambda_{graphene}\) depends on the strain modulus of the substrate, with stiffer surfaces producing smaller wavelengths. The Young’s modulus and Poisson’s ratio of graphene are \(E_g = 1\) TPa and \(\nu_g = 0.165\), respectively. Equation 1 includes a prestretch factor \((S)\), corresponding to the prestretched substrate:

$$S = (1 + (1 + e)^2)/2(1 + e)$$

The average amplitude of the wrinkles is

$$A = \frac{L t}{\lambda} \sqrt{\frac{8u}{3(1 - \nu)^2}}^{1/2}$$

From FESEM image analysis, the prestretch factor, \(S\) is calculated to be 2.657 (see the Supporting Information, Section 3). Here, \(A\) is the amplitude of the wrinkles and \(L\) is the average length of the wrinkles. The perpendicular stress on the graphene can be estimated by

$$P = \frac{wE_S}{2\pi} \frac{w_{max}}{3SE_S}^{1/3}$$

where \(w\) is the perpendicular displacement of the graphene and \(P\) is the stress component by the bacterial cell wall acting perpendicular on graphene (see the Supporting Information).

Combining eq 1 with the experimental wavelength values, we estimate Young’s modulus of the bacterial cell wall to be between 33.7 and 42.2 MPa, comparable with other measurements (39 MPa).54 Further, we calculate the average amplitude of wrinkles as \(A = w_{max} = 7.3\) nm from eq 3, which is also consistent with AFM measurements, and the maximum perpendicular stress \((P_{max})\) acting on graphene is 40.18 MPa. Further studies are required to understand the effect of other bacteria, such as Gram-negative bacteria. Since for one bacterial cell, the Young’s modulus would not change, the wrinkle wavelength is fixed for one cell. The wavelength can be tuned by changing the cell type. The amplitude of the wrinkle is dependent on the shrinkage of the cell. This too can be changed by changing the cell. Therefore, the amplitude or wavelength of wrinkles can be tuned if we use different cell types. Further, graphene wrinkles can be compared with wrinkles on raisins, \(\lambda = 1.5\) mm (Figure 1), where with the grape skin being 30 times stronger than the grape pulp implies a skin thickness of 111 μm. Remarkably, graphene, being ~370,000 times thinner than grape skin, is able to retain its mechanical characteristics while interacting with the shrinking bacterium.

CONCLUSIONS

In summary, the bacterium scaffold can be employed to achieve selectively patterned, aligned, confined, and electrically anisotropic graphene wrinkles. This is realized by flap-valve operation of graphene, which functions as a mass-transfer diode. The longitudinal (high-texture aspect ratio) graphene wrinkles with ultrasmall wavelength (32–34 nm) controllably aligned between electrodes exhibit an anisotropic transport barrier \((\Delta E = 1.69\) meV\). This study can be extrapolated for formation of confined wrinkles on other 2DNs and for reduced wrinkle wavelengths via bacterial cells with a higher Young’s modulus (or via hygroscopic polymer patterns). The work could also evolve advances in cytoelectronics and 2D electronic circuitry with controlled wrinkle placement.

MATERIALS AND METHODS

Experimental Process. Bacteria Preparation. Rod-shaped, Gram-positive Bacillus subtilis bacterial strands (0.5 to 1.5 μm in width and lengths from 1 to 5 μm) were used in this study. B. subtilis were grown in agar gel (OXOID CM0003B), and care was taken to ensure that there is no cross-contamination. A pellet of B. cereus cells was introduced into 100 mL of nutrient broth solution (0.13 g/mL) and sterilized with a cotton seal and placed in an incubator to grow the culture at 31.0 °C for 14–15 h (shake frequency = 62 rpm). After that, the bacterial cells were separated from the medium by centrifuging the suspension at 6000 rpm for 10 min and resuspending the pellet in DI water. This was repeated three times to remove the nutrient broth from the bacterial suspension.

Bacteria Deposition on the Silicon Dioxide Surface. A fresh chip (285 nm SiO₂-on-silicon substrate) was sequentially washed with...
acetone, isopropyl alcohol, and DI water and was dried under N₂ flow. The prewashed chip was exposed to oxygen plasma (0.0058 psi, 100 W, 2 min). The chip was then immersed in the bacterial suspension (in DI water) for about 1 h, followed by carefully washing and drying in N₂ flow. This leads to bacterial cells adhering on the SiO₂ substrate by excreting extracellular polysaccharides, which bind them on the surface. Because of washing by DI water, the polysaccharides will not exist on the top surface of the bacteria. The process is shown in Figure S1. **Graphene Growth and Transfer.** In this step, a high-quality monolayer graphene sheet is laid on top of the bacterial cells. Here, graphene is grown via a CVD process on a copper foil (25 μm) at 1000 °C with purging CH₄/H₂ (20/10 sccm) in a 1 inch quartz tube for 5 min. A layer of PMMA is then spin-coated (20% PMMA at 4000 rpm for 1 min) on graphene (on Cu foil), followed by dissolving the copper foil in 30% nitric acid. The PMMA–graphene composite film that floats on the top was transferred to a water bath to get rid of the acid residues. The film is then carefully transferred onto the bacterial chip prepared in the previous step. This is followed by the removal of the PMMA layer (acetone solution wash, 50 °C for 4–5 min) to produce graphene-wrapped bacteria immobilized on the chip. **Annealing Process.** Here, the sample was exposed to vacuum and high temperature to trigger the wrinkle formation process. For this, the first, the CVD tube was flushed with H₂ gas (100 sccm) for 15 min. The chip (from step 3 having bacteria covered by graphene) was placed into the vacuum chamber and the temperature was raised to 250 °C. The bacterial cells experience a force toward positive field gradient (higher field intensity) and thus are directed toward the electrode junction, where they immobilize, bridging the electrode gap. This is followed by transfer and placement of monolayer graphene atop the bacterial cells. **Photolithography and Device Fabrication.** Photoresist was spin coated on the samples at 4000 rpm for 40 s, followed by soft baking at 110 °C for 1 min. Samples are aligned and exposed in an MA6Mask aligner with hard contact for 12 s at 900 W UV power. The exposed samples were developed in developer for 12 s. O₂ plasma exposure was performed on this developed samples to remove the uncovered graphene, followed by immersion in etcher for 5 min. Finally, samples were washed and cleaned in DI water. **Simulation Processes and Parameters.** The adhesion energies of the hybrid system, isolated graphene monolayers, and lipid bilayers, respectively. The value of γₛ,ₘ 288 mJ m⁻² for the graphene–silica substrate contact is taken from the literature. The adhesion between bacteria and substrate, which is less relevant to the current problem, is set to the same value for simplicity. **Extended Finite Element Method Calculations.** There is some air remaining under the graphene sheet before annealing, and at 250 °C, some of the organic composition degenerates and releases some gases, such as CO₂, NH₃, and SO₂. Assuming the gas released under vacuum is 1% of the mass of the total liquid phase, the pressure increase under the vacuum environment is from the released gas and vapor steam from water. The volume of the liquid phase (water, bacteria, and other organic compositions) is Vₗ, and the volume of air is Vₐ before annealing. Assume the ratio of the liquid phase to air is ϵₗ/Vₑₐ = 10. The molecular number of the gas phase is nₛ = VₛDₛ/Mₛ, the molecular number of the gas phase is nₗ = VₗDₗ/Mₗ, Dₛ is the density of air, which is 1.27g/L. Dₛ is the density of the liquid phase; we set it to be the same as water’s, which is 1000 g/L. Mₛ and Mₗ are
molecular masses of the gas and released gas mixture; both of them are 29. The volume of the total gas phase (\(V_g\)) may increase twice under vacuum before crack formation compared with that (\(V_A\)) in atmospheric pressure (\(P_A\)).

\[
\frac{n_A}{V_A} = \frac{D_A}{M_A}
\]

\[
\frac{n_L}{V_L} = \frac{D_L}{M_L}
\]

\[
m_g = V_L D_L \times 0.01
\]

\[
g_g = V_L D_L \times 0.01 / M_g
\]

Applying \(PV = nRT\),

\[
P = \frac{nRT}{V}
\]

\[
P_A = \frac{n_A V_A T_A}{n_L V_L T_L} = \frac{100 \times 525}{2 \times 1.27 \times 298} = 69
\]

The partial pressure of water vapor is still about 40 bar, because the concentration of the soluble content in water is very low at this temperature (Raoult's law). Therefore, the total pressure could be 69 + 40 = 109 bar.

To characterize the crack nucleation and development of the graphene under interior gas pressure, we adopt the extended finite element method to simulate the growth of the crack in the graphene. XFEM is a numerical technique that extends the classical finite element method (FEM) approach by extending the solution space for solutions to differential equations with discontinuous functions. A key advantage of XFEM is that in such problems the finite element mesh does not need to be updated to track the crack path, while FEM has to use mesh refinement to solve such problems.

We first construct the graphene–cell–SiO\(_2\) system shown in Figure 4a and equilibrate the system using molecular dynamics simulation. To determine where the crack will be nucleated under interior gas pressure, we can apply a uniform pressure on graphene, as shown in Figure 4b. From the FEM calculation results, we find that the location where curvature of the height profile is zero bears the maximum principle stress. We then simulate the evolution of the crack using XFEM; the crack evolution at different time points is shown in Figure 4c. To demonstrate the growth of the crack, we also plot the PHILSM (the level set value of friction angle) contour of the crack tip at different time points. The movie showing the development of the crack can be found in Supporting Movie S4.

**Atomic Molecular Dynamics Simulations (AMD).** These were performed with NAMD\(^{26}\) and the CHARMM force field (CHARMM general force field, CHARMM36 lipid force field),\(^{47-49}\) using the Langevin dynamics (time step of 2 fs, temperature of \(T = 300\) K, and damping coefficient of \(\gamma_{long} = 0.1\) ps\(^{-1}\)). Nonbonding interactions had a cutoff distance of \(d = 10\) Å, and long-range electrostatic interactions were calculated by the PME\(^{20}\) method (periodic boundary conditions). In Figure 4d, we show the stabilized atomic level structure of the wrinkled graphene on the bacterial cell obtained after 2 ns of steered MD simulations. First, we used CHARMM-GUI\(^{15-16}\) to prepare the lipid bilayer (80 × 550 Å\(^2\)) composed of two different types of bacterial phospholipids, PMPE (neutral) and PVPG (negatively charged), in a 2:3 ratio, respectively. We have also prepared the peptidoglycan layer (thickness 25–30 Å, 100 × 600 Å\(^2\)). The layer was composed of linear chains of two amino sugars, namely N-acetylmuramic acid (GlcNAc) and N-acetylmuramuramic acid (MurNAc). MurNAc was joined with an amino acid chain containing alanine, glutamine, lysine, and alanine, and these tetrapeptides were interbridged with five glycin molecules. We have separately equilibrated (5 ns) in an NPT ensemble the hydrated lipid bilayer and the peptidoglycan layer with a graphene layer adsorbed on its top. Then, we have combined these subsystems and modeled them as a whole with steered MD simulations (forced), while applying a damping coefficient of 50 ps\(^{-1}\). The force was applied on all atoms except hydrogen. Its value was proportional (\(k = 0.1\) pN) to the square of the distance from the system center (length 600 Å). The force was acting from the outer surface (graphene) toward the inner surface (lipid bilayer) of the cell wall, and at each point its direction was normal to the graphene surface.

**ASSOCIATED CONTENT**

**Supporting Information**

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Section 1. Calculation of average wavelength from AFM image; Section 2. Calculation of average wavelength from FESEM images; Section 3. Calculation of average wavelength and average amplitude in model; Section 4. Calculation of Strain in graphene from 2D peak shifts in Raman spectroscopy (PDF)

Graphene flap-valve and space bag (MOV)

The graphene coating process on a bacteria lying on the substrate (MOV)

Wrinkle development in the graphene–bacteria contact after the bacteria shrinks (MOV)

XFEM for crack formation process (MOV)

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**Author Contributions**

V.B. conceived and directed the project, S.D. conducted most of the experiments, Z.X., E.G., and Y.W. conducted coarse-grained simulations, while P.K. and S.S. performed molecular dynamics simulations. S.D., T.S.S., and S.B. performed characterizations. All authors contributed toward writing the manuscript.

**Notes**

The authors declare no competing financial interest.

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