Adhesive Characteristics of Low Dimensional Carbon Nanomaterial on Actin

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Abstract: The biosafety of carbon nanomaterial needs to be critically evaluated with both experimental and theoretical validations before extensive biomedical applications. In this letter, we present an analysis of the binding ability of two-dimensional monolayer carbon nanomaterial on actin by molecular simulation to understand their adhesive characteristics on F-actin cytoskeleton. The modelling results indicate that the positively charged carbon nanomaterial has higher binding stability on actin. Compared to crystalline graphene, graphene oxide shows higher binding influence on actin when carrying positive surface charge. This theoretical investigation provides insights into the sensitivity of actin-related cellular activities on carbon nanomaterial.

Keywords: Actin, Graphene, Graphene oxide, Adhesion

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Inorganic materials are widely applied in biomedical engineering for tissue regeneration and surgical replacement, therefore critical insight into their biosafety is required before extensive applications in this area of growing need[1]. Among the potential inorganic biomaterials, graphene is a two-dimensional monolayer carbon nanomaterial on which carbon atoms are packed into honeycomb lattice that provides it with exceptional physical and chemical properties[2]. Graphene oxide (GO) is the disordered analogue of crystalline graphene and allows higher interaction with a wide range of organic and inorganic materials because of its oxygen-containing functional groups[3].

Various biomedical applications were proposed based on the physical and chemical properties of graphene and GO[4]. However, experiments have shown that a significant high uptake of graphene nanomaterials by tumor cells occur *in vivo*[5]. GO also exhibits dose-dependent cytotoxicity on both human and animal cells[6]. A recent experiment demonstrated that GO particles can localize on F-actin networks when living cells are cultured in GO solution[7]. Among the various biomacromolecules, actin is the most abundant structural protein in the human body, whose biophysical behaviors can alternate cell cycles by adjusting the mechanical behaviors of living cells[8,9]. Therefore, the adhesion of low dimensional carbon nanomaterial on F-actin networks can potentially mediate the cellular activities of living cells. It is therefore imperative to understand the adhesive characteristics of graphene/GO on actin for the purpose of reliable biomedical applications.

Experimental techniques have been utilized to investigate the micro/nanoscale interactions between living organisms and inorganic materials. For example, atomic force microscopy has been used to characterize the adhesion between living cells and an inorganic substrate[10]. However, the inorganic particles to which in cells attach are often nanoscopic in size, thereby rendering it difficult to perform physical or experimental characterization involving the micromanipulation of proteins to gain quantitative insight. In combination with experimental characterization, molecular modeling provides a powerful tool for probing the mechanism of the interaction between organic macromolecules and inorganic materials. Systematic molecular dynamics (MD) exploration have been conducted to investigate the mechanical properties carbon nanomaterials. The small scale vibrational characteristics of multi-walled carbon nanotube have been investigate with respect to elastic and thermal properties[11,12]. The failure properties of carbon nanotube and graphene have been further explored insightfully[13,14]. Moreover, the mechanical instability of carbon nanomaterials have been studied at nanoscale[15,16]. Despite these numerical explorations of the mechanical behaviors of carbon nanomaterials, their adhesive characteristics on biomolecules need further theoretical studies. *ab initio* and MD modeling strategies have been applied to characterize the interaction between biomolecules (e.g. protein, amino acids and nuclei acids) and inorganic materials (i.e. graphene and hydroxyapatite)[17,18]. However, the computational investigation is very limited in understanding the mechanisms underlying the interfacial relationships between actin and an inorganic material, which is important for gaining insights of how inorganic biomaterials sensitively mediate cytoskeleton-related cellular activities in biological environments.

This letter presents a modelling technique that can assist the study of the physics of the interaction between actin and graphene/GO based on MD simulation approach. Different charge states of carbon atoms on graphene/GO are studied leading to an understanding of the sensitivity of cell adhesion to atomic charge states. This molecular level investigation provides insight that can elucidate the nature of the physical events occurring at the rigor binding sites between monolayer carbon nanomaterial and F-actin cytoskeleton.

G-actin is the macromolecular monomer of F-actin cytoskeleton that is adopted in the biophysical model presented; where the 2ZWH G-actin model[19] is selected as the starting condition in our approach. A square graphene nanomaterial is developed with armchair boundaries and an edge length of 18 nm. The general simulation model is presented in Fig. 1(a); the graphene/GO layer is located above the actin and approaches it during simulation/sampling. To start with, geometry minimization was performed for each macromolecule's configuration in vacuum to ensure dimensional reliability for the MD simulation. After geometry optimization, 100 ps relaxation was first performed in canonical (NVT) ensemble by using Berendsen method[20], with the temperature maintained at 303 K. Subsequently, another 100 ps relaxation was performed in isothermal-isobaric (NPT) ensemble at a constant pressure of 1 atm by using Parinello-Rahman method [21]. A dynamically equilibrated configuration of the molecular system can be obtained for later sampling simulations after these relaxation processes. Particle mesh Ewald (PME) method is adopted to calculate the coulomb potentials, and the cut off radius for van der Waals interaction is 2.0 nm. Umbrella sampling was performed with the stable molecular configuration obtained from aforementioned relaxation process. The sprint constant for umbrella sampling is 1000 kJ/mol·nm^2. The OPLS-AA force field[22] was utilized in the MD simulation and implicit solution strategy[23] was adopted to limit the computational cost of MD simulations. All MD simulations were finished using Gromacs[24], with molecular visualization conducted with VMD[25]. The time step for all MD simulations is 2 fs and the modeling time for umbrella sampling is 1 ns. We note that the PME in the calculation of coulomb potentials significantly increased the computational cost. However, this treatment is important to the characterization of long-distance electrostatic interaction that is crucial to the phenomenon of biosorption.

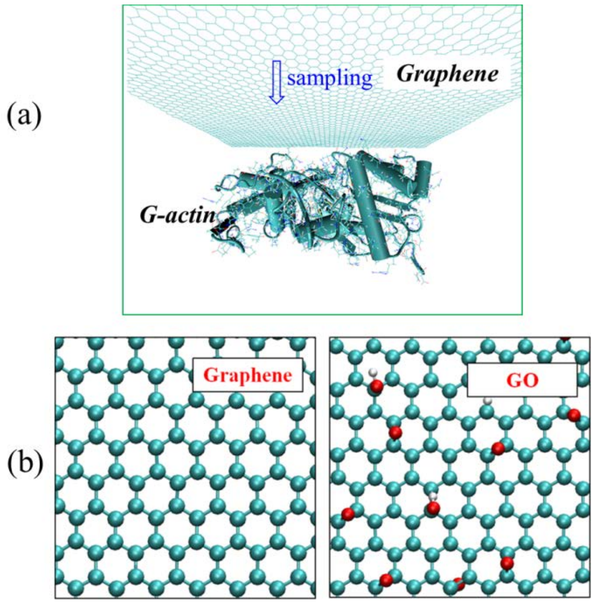

FIG. 1. Molecular modelling details. (a) General MD simulation model that consists of monolayer graphene and single G-actin monomer. (b) Molecular conformation of graphene and GO. Cyan dots denote carbon, red dots denote oxygen and silver dots denote hydrogen.

The non-bonded interaction energy between two sub-groups (graphene and G-actin) was extracted from the MD simulation trajectory results. This interaction energy denotes weak chemical interactions, which mainly includes van der Waals interaction, electrostatic interaction and H-bond interaction. According to the principle of minimum potential energy, molecular configuration with lower interaction energy corresponds to higher binding stability in the process of adhesion.

Oxidization of graphene can result in various functional groups that might change the physical properties of monolayer carbon nanomaterial. In order to study how these functional groups can change the adhesive characteristics of carbon nanomaterial on G-actin, we also designed a molecular structure of monolayer honeycomb carbon materials in association with oxygen-containing functional groups (i.e. epoxy, C-OH and C-COOH), as is shown in Fig. 1(b).

The interaction energy profiles between G-actin and graphene/GO are provided in Fig. 2. When the distance between graphene and G-actin is about 1.41 nm, the potential energy is minimum, which indicates the highest binding stability of graphene on G-actin. For GO structure, the minimum interaction energy corresponds to a distance of 1.55 nm, which is larger than the distance for graphene. This difference in distance is arguably due to the additional oxygen-containing functional groups on the surface of honeycombed carbon lattice.

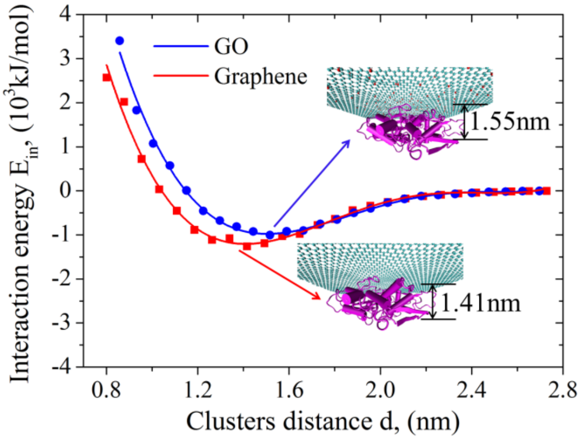

FIG. 2. Interaction energy between actin and carbon nanomaterials with respect to distance between nanomaterial and G-actin

When approaching the carbon nanomaterial, the additional oxygen containing groups would provide van der Waals forces to resist this approaching body. Therefore, compared to graphene, the saturated binding energy of GO occurs at a further distance from the actin. Therefore, the degree of van der Waals interaction from the carbon nanomaterial on the G-actin will be reduced.

According to chemical theories, the adherence characteristic of an inorganic material with protein in the biological environment is a function of the interaction forces between protein molecules and the surface of the inorganic material. Also the electrostatic force plays a critical role in the adhesion between protein and inorganic materials[26]. Experimental findings have verified that many biological attachments such as cell attachment and drugs binding are pH sensitive[27], as the cellular pH value can alter the electrostatic states of protein in biological environments. The normal pH value for living cells to survive in the human body is 7.4 and the isoelectric point of actin is 4.8[28], which makes actin usually negatively charged. It is presumable that when exposed to positively charged carbon materials, actin would present higher absorption ability due to the strong electrostatic interaction.

Recent experimental findings demonstrate that the surface charge of graphene can be mediated by controlling the chemical component of the surrounding organic solution[29]. Similarly, GO can be either positively or negatively charged according to the chemical conditions[30]. It can therefore be argued that studying neutral carbon nanomaterial alone would not produce a full insight on biological adhesion. Consequently, we have developed a model that accounts for the influence of the charge condition of carbon atoms on biological adhesion. The biological environment in human body for living cells to survive is quite complex and can therefore lead to different charge states of the carbon atoms in carbon nanomaterials, leading to the conclusion that the charge carried by a carbon atom is significant in determining the nature of the contact between G-actin and graphene/GO substrates. Based on the potential charging characteristics, we have considered numerical modeling scenarios, in which the charge of each carbon atom ranges from -0.1*e* to +0.1*e*. The interaction force results for graphene in different charge states are provided in Fig. 3(a).

When the graphene is negatively charged, the interaction force between G-actin and graphene is smaller compared to positively charged nanomaterials, indicating that the crystalline graphene is more difficult to be localized on F-actin networks (whose principal component is actin), because they are repel the negatively charged protein as they approach the surface. For positively charged carbon atoms, the graphene shows higher attractive force on G-actin, indicating the propensity for higher binding on F-actin cytoskeleton. Similar characteristics have been found in the study of interaction force between F-actin and positively charged lipids membrane[31].

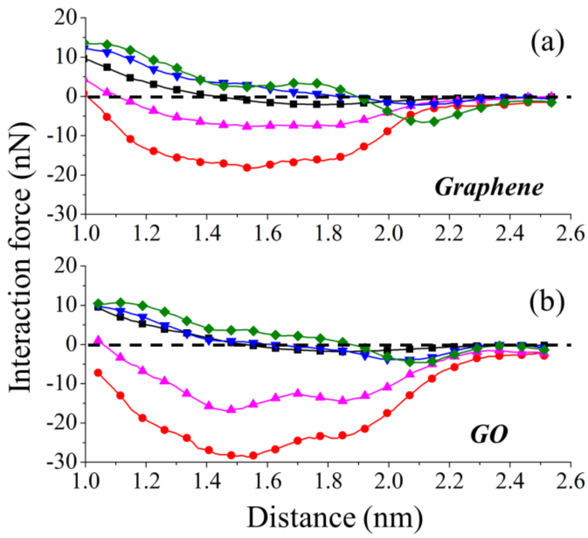

FIG. 3. Interaction force between G-actin and graphene/GO relative to carbon atom charge state. In both figures, red circle, magenta up triangle, black square, blue down triangle and olive diamond respectively denotes the charge states from +0.1*e*, +0.05*e*, 0*e*, -0.05*e* and -0.1*e*. Negative force means attracting while positive force means repelling.

The interaction force between GO and G-actin is also extracted for different carbon atom charge states and the corresponding interaction force profiles are provided in Fig. 3(b). Similar to crystalline graphene, positively charged carbon material also shows higher binding ability on actin compared to negatively charged material. Moreover, the binding stability increases with the positive charge of carbon atom, which indicates that, the GO absorption on F-actin cytoskeleton can be mediated by changing the charge of atoms on carbon nanomaterial.

We further compare the interaction forces between actin, graphene and GO when their carbon atoms are both positively charged (Fig. 4). The larger attractive force obtained from the GO model indicates that GO has a larger chance of localizing on F-actin cytoskeleton than graphene when the carbon atom equally carries positive charge, which is consistent with classic chemical theories[3] and experimental findings[7]. The absorption ability increases with the positive charge of carbon nanomaterial.

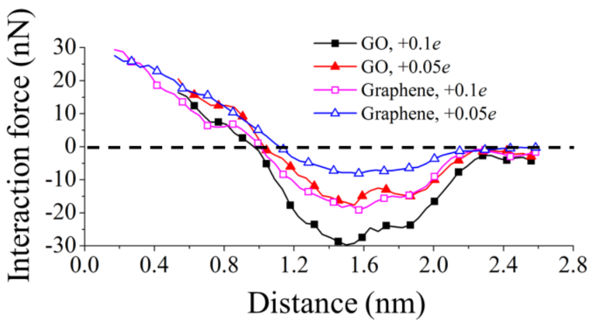

FIG. 4. Interaction force as a function of the distance between G-actin and positively charged GO/graphene

The molecular configuration of GO binding on actin with lowest interaction energy (highest binding stability) is extracted to study the mechanisms of the physical interaction between actin and carbon nanomaterial (Fig. 5). We have tracked only the H-bond and electrostatic interactions subject to the scientific knowledge that van der Waals interaction is much weaker than electrostatic bonding, and that this bond type is always present regardless of whether or not functional groups are involved. Two typical residues (e.g. GLU270 and LYS284) on actin are extracted as examples to investigate typical long range interactions. The nitrogen atom on GLU270 can potentially form an H-bond with the epoxy on carbon lattice. This H-bond interaction can partly contribute to an increase in the interaction force of carbon nanomaterial with the help of oxygen containing functional groups. Moreover, the electrostatic interaction between actin and GO is significantly responsible for the increase in the attraction force. For example, the hydrogen atom in protein residues usually carries a positive charge of 0.06~0.3*e* (i.e. the hydrogen atoms in LYS284), which will effectively increase the force of attraction on negatively charged carbon atoms. The oxygen atom in a functional group also carries a positive charge, which will further increase the interaction force on actin.

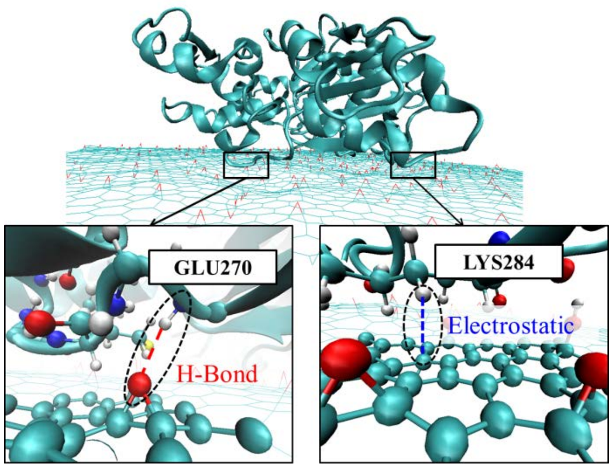

FIG. 5 Physical mechanism of the interaction between GO and actin. Cyan dots denote carbon, red dots denote oxygen and silver dots denote hydrogen.

However, the surface charge of nanomaterial is characterised with uncertainty, especially in respect of the oxygen containing functional groups. Therefore, more investigation about the charge states of functional groups needs to be conducted in future by both experiments and theoretical evaluation to understand the biocompability and biosorption ability of functionalized carbon nanomaterial.

In summary, the interaction between actin and low dimensional carbon nanomaterial has been investigated by molecular modelling method to understand the adhesive characteristics of monolayer carbon nanomaterial (i.e. graphene and GO) on F-actin cytoskeleton. For neutral condition, oxidation on the carbon monolayer can slightly decrease the interaction force of monolayer carbon nanomaterial on actin. The positive charge of carbon atoms on graphene/GO can significantly improve their binding ability on actin, and the binding affinity of carbon nanomaterial is proportional to the positive charge it carries.

The theoretical investigation of interaction mechanisms offers clues that can assist in exploring the biosorption and biocompability of carbon nanomaterial. In the future, with further developed sub-cellular manipulation techniques, accurate characterization of the interaction between graphene/GO and proteins can be conducted to better understand the interaction mechanisms.

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Reference

[1]B. Fadeel and A. E. Garcia-Bennett, Advanced Drug Delivery Reviews **62,** 362 (2010).
[2]S. Stankovich, D. A. Dikin, G. H. B. Dommett, K. M. Kohlhaas, E. J. Zimney, E. A. Stach, R. D. Piner, S. T. Nguyen, and R. S. Ruoff, Nature **442,** 282 (2006).
[3]K. P. Loh, Q. Bao, G. Eda, and M. Chhowalla, Nat Chem **2,** 1015 (2010).
[4]X. Hu and Q. Zhou, Chemical Reviews **113,** 3815 (2013).
[5]K. Yang, S. Zhang, G. Zhang, X. Sun, S.-T. Lee, and Z. Liu, Nano Letters **10,** 3318 (2010).
[6]K. Wang, J. Ruan, H. Song, J. Zhang, Y. Wo, S. Guo, and D. Cui, Nanoscale Res Lett **6,** 8 (2011).
[7]M.-C. Matesanz, M. Vila, M.-J. Feito, J. Linares, G. Gonçalves, M. Vallet-Regi, P.-A. A. P. Marques, and M.-T. Portolés, Biomaterials **34,** 1562 (2013).
[8]G.-K. Xu, X.-Q. Feng, H.-P. Zhao, and B. Li, Physical Review E **80,** 011921 (2009).
[9]Y. Li, G.-K. Xu, B. Li, and X.-Q. Feng, Applied Physics Letters **96,** 043703 (2010).
[10]L. Benoit and N. Alice, Reports on Progress in Physics **75,** 116601 (2012).
[11]Y. Q. Zhang, G. Liu, and X. Han, Physics Letters A **340,** 258 (2005).
[12]Y. Q. Zhang, X. Liu, and G. R. Liu, Nanotechnology **18,** 445701 (2007).
[13]Z. Qin, Q.-H. Qin, and X.-Q. Feng, Physics Letters A **372,** 6661 (2008).
[14]T. Zhang, X. Li, S. Kadkhodaei, and H. Gao, Nano Letters **12,** 4605 (2012).
[15]Y. Q. Zhang, G. R. Liu, and J. S. Wang, Physical Review B **70,** 205430 (2004).
[16]Y. Q. Zhang, G. R. Liu, and X. Han, Physics Letters A **349,** 370 (2006).
[17]A. Rimola, M. Corno, C. M. Zicovich-Wilson, and P. Ugliengo, Journal of the American Chemical Society **130,** 16181 (2008).
[18]K. Jin, X. Feng, and Z. Xu, BioNanoScience **3,** 312 (2013).
[19]T. Oda, M. Iwasa, T. Aihara, Y. Maéda, and A. Narita, Nature **457,** 441 (2009).
[20]H. J. C. Berendsen, J. P. M. Postma, W. F. Van Gunsteren, A. DiNola, and J. Haak, The Journal of Chemical Physics **81,** 3684 (1984).
[21]M. Parrinello and A. Rahman, Journal of Applied Physics **52,** 7182 (1981).
[22]W. L. Jorgensen, D. S. Maxwell, and J. Tirado-Rives, Journal of the American Chemical Society **118,** 11225 (1996).
[23]D. Qiu, P. S. Shenkin, F. P. Hollinger, and W. C. Still, The Journal of Physical Chemistry A **101,** 3005 (1997).
[24]D. Van Der Spoel, E. Lindahl, B. Hess, G. Groenhof, A. E. Mark, and H. J. C. Berendsen, Journal of computational chemistry **26,** 1701 (2005).
[25]W. Humphrey, A. Dalke, and K. Schulten, Journal of Molecular Graphics **14,** 33 (1996).
[26]K. Yamazaki, T. Ikeda, T. Isono, and T. Ogino, Journal of Colloid and Interface Science **361,** 64 (2011).
[27]S. K. Vashist, D. Zheng, G. Pastorin, K. Al-Rubeaan, J. H. T. Luong, and F.-S. Sheu, Carbon **49,** 4077 (2011).
[28]J. H. Collins and M. Elzinga, Journal of Biological Chemistry **250,** 5915 (1975).

[29]W. W. Liu, J. N. Wang, and X. X. Wang, Nanoscale **4,** 425 (2012).
[30]H. Zhang, X. Zhang, D. Zhang, X. Sun, H. Lin, C. Wang, and Y. Ma, The Journal of Physical Chemistry B **117,** 1616 (2012).
[31]R. Grimard, P. Tancrede, and C. Gicquaud, Biochemical and Biophysical Research Communications **190,** 1017 (1993).