Ovalbumin-mediated synthesis and simultaneous functionalization of graphene with increased protein stability

Riaz Ullah\textsuperscript{a}, Shadab Ali Khan\textsuperscript{b}, Aref Ali Mohammed Aladresi\textsuperscript{c}, Sulaiman Ali Alharbic and Arunachalam Chinnathambi\textsuperscript{c}

\textsuperscript{a}Medicinal, Aromatic and Poisonous Plants Research Center (MAPRC), College of Pharmacy, King Saud University, Riyadh, Saudi Arabia; \textsuperscript{b}Biochemical Sciences Division, CSIR-National Chemical Laboratory, Pune, India; \textsuperscript{c}Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, Saudi Arabia

ABSTRACT
In this article, we have shown an easy, one-step, protein-directed approach for the synthesis of water-soluble and functional graphene using ovalbumin. The obtained ovalbumin functionalized graphene was characterized by UV–visible spectroscopy, X-ray diffraction, Raman spectroscopy, and Fourier transform infrared spectroscopy. Atomic force microscopy was used to check the attachment/functionalization of ovalbumin on graphene sheets. It was shown that the functionalization of ovalbumin on graphene sheets makes the protein more thermally stable compare to free ovalbumin, which has been shown by using sodium dodecyl sulfate polyacrylamide gel electrophoresis.

ARTICLE HISTORY
Received 28 July 2019
Accepted 19 December 2019

KEYWORDS
Ovalbumin; graphene oxide; graphene; Atomic force microscopy; SDS-PAGE; thermal degradation

1. Introduction
Graphene synthesis has gained tremendous interest in the scientific community in recent times (1,2). Recently, most of the researchers have been obtaining graphene oxide using natural graphite powder, and this chemically derived graphene oxide is being reduced chemically for the bulk production of graphene. Graphene oxide is hence being used for efficient, cost-effective, and large-scale synthesis of graphene and its derivatives (3). The π–π stacking interactions present in graphene make it vulnerable for aggregation, and hence, to improve the stability and to make it functional chemical modifications of graphene have become necessary (4,5). Noncovalent methods for modifying graphene are always better compared to covalent bonding-based methods such as simplicity and reversibility of the attachment process with low risk of permanently changing its basic properties and structures (6,7). Synthesis of graphene has been achieved by various physico-chemical methods such as mechanical/ultrasonic exfoliation and chemical vapor deposition (1,2). Compared to other methods, chemically reduced graphene oxide has high scope for functionalization and mass scale production (8–10). However, the production of graphene in this way has certain limitations such as the use of toxic agents like hydrazine, which is being employed for reducing graphene oxide chemically. Apart from this, not only the obtained hybrids are hydrophobic but also not recommended for biological applications, where water-loving materials are required. Hence, these protocols may prove expensive and ecounfreindly when mass production is required. Hence, the onus is on researchers to find alternate protocols for the production of graphene from graphene oxide. Various green reducing agents such as vitamin C (11–13), amino acid (12), reducing sugar (14), microorganisms (15–17), and plant extracts (18,19) are being...
employed to obtain graphene from graphene oxide. Researchers are continuously striving to find novel approaches for the effective conversion of graphene oxide into functional and water-soluble graphene. Toward this end, Wang et al. have reported aromatic rings of tea polyphenol (TP) present in a tea solution as effective reducing agents for the reduction of graphene oxide (20).

Proteins are biological polypeptides that are made up of both hydrophilic and hydrophobic amino acids. The presence of these amino acids on protein surfaces makes them favorable to attach or form bonds on solid surfaces (21,22), considering this one could exploit this property of proteins to adsorbed them on basal planes of graphene sheets. Liu et al. have reported bovine serum albumin (BSA)-based, environment friendly one-pot reduction method to obtain BSA-conjugated graphene composites, which was shown to be soluble depending on pH and demonstrated that the attachment of BSA as a “universal glue” makes graphene oxide and graphene available for the successful and efficient functionalization of nanoparticles having different properties such as size, shape, compositions, and surface chemistries (23).

Chicken egg albumin (ovalbumin), a phosphorylated glycoprotein, constitutes the major part of egg white. It has a molecular weight of 45 kDa and possesses 385 amino acid residues (24). Ovalbumin can be used as a carrier protein to conjugate to synthetic peptides for use as an immunogen. It contains 20 Lys, 10 Tyr, 6 Cys, 14 Asp, and 33 Glu amino acid residues, which makes it suitable for conjugation on substrates.

Here, we have demonstrated a simple and eco-friendly protocol for the one-step reduction and simultaneous functionalization of graphene sheets using chicken egg albumin or ovalbumin. We have also shown that the immobilization of ovalbumin on graphene sheets has increased its stability and make it more stable toward thermal degradation compare to free ovalbumin.

2. Experimental

2.1. Materials

Graphene oxide was purchased from Graphene Laboratories Inc. (USA), and ovalbumin from chicken egg white (Grade V) was purchased from Sigma Aldrich (USA). All reagents were prepared using double distilled water. All other chemicals were purchased from Sigma Aldrich.

2.2. Synthesis of ovalbumin reduced graphene

A total of 1 ml of graphene oxide aqueous dispersion (1.0 mg/mL), 1 ml of distilled water, and 1 ml of ovalbumin solution (30 mg/mL) were mixed and vigorously stirred for 5 min. Then, NaOH (0.5 M) was added (to make the pH of the solution to 9), and the mixture was stirred vigorously for another 5 min. The initial light yellow-brown solution of the ovalbumin–graphene oxide mixture was incubated at 55°C for 24 h and after that the color of the solution turns black and becomes stable, indicating the successful formation of the ovalbumin reduced graphene biocomposite. The product was then purified by centrifugation at 12,000 rpm for 20 min. The final purified ovalbumin reduced graphene biocomposite was then redispersed in distilled water for further characterizations.

2.3. Thermal degradation of free ovalbumin and ovalbumin reduced graphene

Free ovalbumin and ovalbumin reduced graphene samples were dissolved in water and boiled for 5, 10, 15, 20, and 25 min separately. After this treatment, these samples were then loaded separately on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) on a slab gel containing 16% (w/v) polyacrylamide by the method of Laemmli, and the gels were stained using silver staining (25).

2.4. Characterization

2.4.1. UV–visible spectroscopy

Perkin Elmer UV-Vis-NIR spectrophotometer (Lambda 750) at a resolution of 1 nm was used for UV–visible spectroscopic measurements.

2.4.2. X-ray diffraction (XRD)

Philips XPERT PRO instrument, which is equipped with X’celerator, was used for X-ray diffraction (XRD) measurements. Iron-filtered Cu Kα radiation (λ = 1.5406 Å) and 121 active channels were used to scan the sample. 5°–50° (2θ range) with a step size of 0.02° and duration of 5 s per step was used to record XRD patterns with a voltage of 40 kV and a current of 30 mA.

2.4.3. Raman spectroscopy

Confocal micro-Raman Lab Ram HR instrument (LabRam HR, Horiba Jobin Yvon, France) was used for Raman spectroscopy analyses with a CCD detector with a 532 nm Ar laser and a Olympus optical microscope having 100x objective mounted on it. Internal silicon reference at 520 cm⁻¹ was used for calibration with a peak resolution of less than 1 cm⁻¹. The spectra were measured from 500 to 2000 cm⁻¹.
2.4.4. Fourier transform infrared (FTIR) spectroscopy
The Fourier transform infrared (FTIR) spectroscopy measurements were performed on Bruker Tensor 27. Diffuse reflectance mode at a resolution of 2 cm$^{-1}$ was used for all analyses.

2.4.5. Atomic force microscopy (AFM)
Atomic force microscopy (AFM) imaging in tapping mode was performed on a [RHK Technologies, USA (SPM 100)].

3. Results and discussion

3.1. UV–visible spectroscopy
Figure 1 represents the UV–visible spectroscopic measurements of graphene oxide (Figure 1(A), curve 1), ovalbumin reduced graphene (Figure 1(A), curve 2), and standard ovalbumin (Figure 1(B)). The absorption peak at 230 nm in graphene oxide spectrum arises because of π–π$^*$ of the C=C, and a broad shoulder at 300 nm arises because of n–π$^*$ transitions of the carbonyl groups (26). When reduced by ovalbumin (Figure 1(A), curve 2), the peak at 230 nm shifted to 266 nm, indicating that the graphene oxide has been reduced to graphene. This shift is mainly because of structural ordering and π-electron concentration and eventually restoration of sp2 carbon and rearrangement of atoms. Similar red shifting of the plasmon peak when graphene oxide has been reduced to graphene has been reported by other biomolecules also (15–17). Figure 1(B) represents the UV–visible spectrum of standard ovalbumin, which shows a characteristic peak of ovalbumin at 277 nm. Inset in Figure 1(A) shows the digital photographs of aqueous solutions of commercial graphene oxide (left side vial) and ovalbumin reduced graphene (right side vial). From this figure, it is clear that ovalbumin reduced graphene is water miscible and can remain stable for more than 2 months. The change in the initial yellow color of commercial graphene oxide to dark black is another indicator that the graphene oxide has been reduced to graphene successfully.

3.2. XRD measurements
Figure 2 shows the XRD patterns of standard graphene oxide (A) and ovalbumin reduced graphene (B). The XRD pattern of standard graphene oxide shows a peak at 10.06°, which corresponds to plane [002] (27). This peak occurs due to the presence of water and generation of oxygen-bearing functional groups between graphite layers. When graphene oxide reduced to graphene by ovalbumin, this peak at 10.06° shifted to 26.0° (Figure 2).
(B)) and has the same plane [002]. This shifting of peak clearly indicates that the oxygen-bearing functional groups have been removed and the reduction of graphene oxide has been achieved. Another peak at 43° is observed in the pattern, which corresponds to plane [001] of graphene. These peak positions and corresponding planes match well with the reported values of graphene (16–21).

3.3. Raman spectroscopy

Figure 3 represents the Raman spectroscopic measurements of standard graphene oxide and ovalbumin reduced graphene. Raman spectroscopic analysis is one of the most critical techniques for characterizing carbon-based materials containing C=C double bonds, which give high Raman intensities (28). The two main features of graphene analyzed by Raman spectroscopy are the G-peak (arises from first order scattering from sp² carbon atoms) and D-peak (arises from breathing mode of K-point phonons of A1g symmetry). The Raman spectroscopic analysis of standard graphene oxide shows the G-band and D-band at 1580 and 1345 cm⁻¹, respectively. In the Raman spectrum of ovalbumin reduced graphene, the G and D bands shifted to 1599 and 1355 cm⁻¹, respectively, indicating size reduction of the in-plane sp² domains. As D-band occurs because of sp² carbon cluster, its higher intensity in ovalbumin reduced graphene compared to standard graphene oxide confirms the presence of more graphene domain and removal of oxygen groups from ovalbumin reduced graphene (29). Similar Raman spectra have been reported when Escherichia coli was used to obtain graphene from graphene oxide (16). The shifting of the D-band has also been shown in the successful functionalization of reduced graphene oxide using Baker’s yeast (30).

3.4. FTIR measurements

Figure 4 represents the FTIR spectra of standard graphene oxide and ovalbumin reduced graphene. The FTIR spectrum of graphene oxide shows the presence of sharp bands at 1734 cm⁻¹ (for C=O stretching), 1620 cm⁻¹ (for C=C stretching), 1224 cm⁻¹ (for O=C–O stretching), 1054 cm⁻¹ (for C–O stretching), and 3421 and 1385 cm⁻¹ for hydroxyl group deformation peak. These band positions indicate the presence of oxygen containing moieties such as carbonyl, carboxylic, epoxy, and hydroxyl in graphene oxide (31,32). The FTIR spectrum of ovalbumin reduced graphene shows a drastic decrease in intensities of all oxygen functionalities peaks at 1734, 3421, and 1385 cm⁻¹, which corresponds to C=O stretching and OH deformation vibration, respectively. The intensity of the peak at 1224 cm⁻¹, which corresponds to epoxy groups, also decreases in the ovalbumin reduced graphene spectrum. It is important to note that the C–O (alkoxy) stretching peak at 1054 cm⁻¹ has decreased significantly, which shows the excellent potential of ovalbumin in reducing graphene oxide. The emergence of new peaks at 2854 and 2923 cm⁻¹ can be assigned to –CH and –CH₂ stretching vibrations (33) of amino acids present in ovalbumin. The FTIR spectrum of ovalbumin reduced graphene also shows the peaks at 1635 and 1569 cm⁻¹, which represents amide I (mainly for C=O stretching) and amide II bands (N–H stretching), respectively (34). These peaks confirm that the amine group present in the amino acids of ovalbumin reacts with epoxy groups of graphene oxide and confirm that oxygen functionalities have been removed from the surface of graphene oxide.
oxide and results in the formation of ovalbumin functionalized graphene.

3.5. AFM measurements

To further confirm the functionalization (adsorption) of ovalbumin on the graphene oxide surface, AFM analyses were carried out. Figure 5 represents the AFM images of standard graphene oxide and ovalbumin reduced graphene biocomposites and their corresponding height profiles. Figure 5(A) represents the AFM image of standard graphene oxide. From the height profile image, it is clear that the graphene oxide exists in monolayer sheets with a thickness of 1.1 nm, which supports the reported value of single-layer graphene oxide (35). Figure 5(B) confirms the formation of ovalbumin reduced graphene biocomposites. The platelets showed a uniform thickness of about 2.3 nm, which is larger than the thickness of standard graphene oxide, which is about 1.1 nm. These results indicated that ovalbumin adhered uniformly to the graphene oxide sheets after the reduction reaction and slightly increased the thickness of graphene oxide. This type of increase in thickness in AFM analyses after the functionalization of biomolecules on graphene oxide has been reported by several researchers (23,36). However, the ovalbumin reduced graphene still retained the features of single-layer sheets. Hydrophobic and π–π stacking interactions are mainly responsible for the adsorption of protein onto graphene oxide (23). Apart from these interactions, hydrogen bonding between amino acids present in ovalbumin and the functional groups of oxygen present in graphene oxide might have contributed to such attachment.

3.6. Probable mechanism for reduction of graphene oxide into graphene by ovalbumin

Ovalbumin contains aromatic and hydrophobic groups from tyrosine, tryptophan, and hydrophobic amino acids, which are believed to conjugate with the graphene oxide surface through hydrophobic interaction and π–π stacking. The reducing ability of ovalbumin is mainly due to phenolic groups of Tyr residues, which reduces graphene oxide into graphite and themselves converts into quinone groups (37). Ovalbumin also contains many charged hydrophilic residues that help it to dissolve readily in water. Since ovalbumin has both hydrophobic and hydrophilic groups, it can be easily adsorbed on the surface of reduced graphene oxide due to hydrophobic and π–π interaction, making the water-loving groups more aligned with the aqueous

Figure 5. AFM analysis of standard graphene oxide (A) and ovalbumin reduced graphene (B).
phase. This causes the reduction in the surface energy of the obtained graphene sheets, and thus, the resultant ovalbumin reduced graphene remains stable in water.

3.7. Thermal stability of ovalbumin reduced graphene

Ovalbumin reduced graphene biocomposites along with free ovalbumin samples were boiled for 5, 10, 15, 20, and 25 min separately. These samples were then loaded separately on 16% SDS-PAGE. In Figure 6(a) (free ovalbumin) and Figure 6(b) (ovalbumin reduced graphene), lane 1–6 contain standard ovalbumin, and samples were boiled for 5, 10, 15, 20, and 25 min respectively. From Figure 6(a), it is obvious that the free ovalbumin samples give a single band of ovalbumin at 45 kDa only at 10 min of boiling and after that it gets degraded, which can be confirmed by the occurrence of several bands after 10 min (Figure 6(a), lane 4–6) in electrophoretic profile. However, the ovalbumin reduced graphene samples were stable up to 20 min of boiling and give a single band of ovalbumin at 45 kDa (Figure 6(b), lane 2–5). After 25 min of boiling, they also get degraded and result in several bands (Figure 6(b), lane 6) confirming their thermal degradation. This experiment has definitely proved that the functionalization (immobilization) of ovalbumin on graphene sheets has increased its stability against thermal degradation compared to its free counterparts to a certain extent. These observations also suggest that the immobilization of ovalbumin on graphene plays a role in the stabilization of the secondary and tertiary structure and prevents the unfolding of the polypeptide chain of ovalbumin and thereby ultimately making it resistant toward thermal degradation. Zhang et al. have also investigated enzyme immobilization on chemically reduced graphene oxide using horseradish peroxidase (HRP) and oxalate oxidase as model enzymes. They have also concluded that the chemically reduced graphene oxide-enzyme conjugates have high enzyme loading capacity, better activity, and stability than their free counterparts (38).

4. Conclusion

A simple bio-based, environmental friendly, one-pot reduction/functionallization method to obtain ovalbumin reduced graphene nanosheets has been demonstrated. The obtained ovalbumin reduced graphene biocomposites have been shown to be more stable than their free counterparts toward thermal degradation. It was noted that the hydrophobic interactions between ovalbumin and graphene oxide were responsible for such type of adsorption. The type of chemistry between ovalbumin and graphene oxide can also be exploited for other hydrophobic proteins and thus could significantly improve their stability and functions after immobilization. This unique property of graphene and the hydrophobic interaction with biomolecules makes it a potential biomolecules-immobilizing substrate.

Acknowledgements

The authors extend their appreciation to the Researchers Supporting Project number (RSP-2019/110) King Saud University, Riyadh, Saudi Arabia. S.A.K thanks the Council of Scientific and Industrial Research (CSIR), New Delhi for Research Associateship.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

The authors extend their appreciation to the Researchers Supporting Project number (RSP-2019/110) King Saud University, Riyadh, Saudi Arabia.

Notes on contributors

Dr Riaz Ullah is graduated from Kohat University of Science and Technology Pakistan. He worked around 10 year as an assistant.
professor in higher education Pakistan and currently working as a Researcher in Department of Pharmacognosy (Medicinal Aromatic and poisonous plants research center) College of Pharmacy king saud university. His researcher interest is phytoconstituents, biological screening of active ingredients, green synthesis, etc.

Shadab Ali Khan received his M.Sc. degree in Biochemistry from Amravati University, Maharashtra, India in 2003. After completing his masters, he joined Department of Biosciences, National Chemical Laboratory, Pune, India and obtained his Ph.D. degree in 2012 in Biotechnology. After Ph.D, he joined the Department of Chemistry, Indian Institute of Science Education and Research (IISER), Pune, India as Post Doctoral Research Associate. In 2013, he was awarded Post Doctoral Research Associateship by Council of Scientific and Industrial Research (CSIR), New Delhi, India at Department of Biosciences, National Chemical Laboratory. In 2017, he joined Jay Research and Biotech India Private Limited, Pune as Senior Research Scientist. Currently, he is working as Head (Production and R&D) at the same organization and leading a group of 20 associates. His research interests include nanobiotechnology, green synthesis of functional nanomaterials such as graphene, porous carbon, formulation of nanoparticles and bioproducts.

Aref Ali Mohammed Aladresi is a Ph.D. scholar and has been supported in laboratory work.

Prof. Sulaiman Ali Alharbi is a Professor of Bacteriology at the King Saud University, Riyadh. He is an expert in bacteriology research and has published more than 150 research papers in multidisciplinary journals, and he also presented several research topics at international conferences.

Dr. Arunachalam Chinnathambi has completed Ph.D. from Bharathidasan University, India and has got trained in a few molecular techniques from the National University of Singapore and also I got trained in a few techniques of biomaterials and biomolecular sciences at Chung Yuan Christian University, Taiwan. I am a young, energetic, and enthusiastic Microbiologist; I have participated in several international, national conferences, symposia, and presented research papers.

References

[1] Hernandez, Y.; Nicolosi, V.; Lotya, M.; Blighe, F.M.; Sun, Z.; De, S.; McGovern, I.T.; Holland, B.; Byrne, M.; Gun’Ko, Y.K.; Boland, J.J. High-yield Production of Graphene by Liquid-phase Exfoliation of Graphite. Nat. Nanotechnol. 2008, 3, 563–568.

[2] Kim, K.S.; Zhao, Y.; Jang, H.; Lee, S.Y.; Kim, J.M.; Kim, K.S.; Ahn, J.H.; Kim, P.; Choi, J.Y.; Hong, B.H. Large-scale Pattern Growth of Graphene Films for Stretchable Transparent Electrodes. Nature 2009, 457, 706–710.

[3] Lee, C.; Wei, X.; Kysar, J.W.; Hone, J. Measurement of the Elastic Properties and Intrinsic Strength of Monolayer Graphene. Science 2008, 321, 385–388.

[4] Liu, Z.; Robinson, J.T.; Sun, X.M.; Dai, H.J. PEGylated Nanographene Oxide for Delivery of Water Insoluble Cancer Drugs. J. Am. Chem. Soc. 2008, 130, 10876–10877.

[5] Lomeda, J.R.; Doyle, C.D.; Kosynkin, D.V.; Hwang, W.F.; Tour, J.M. Diazonium Functionalization of Surfactant Wrapped Chemically Converted Graphene Sheets. J. Am. Chem. Soc. 2008, 130, 16201–16206.

[6] Patil, A.J.; Vickery, J.L.; Scott, T.B.; Mann, S. Aqueous Stabilization and Self-assembly of Graphene Sheets Into Layered Bio-nanocomposites Using DNA. Adv. Mater. 2009, 21, 3159–3164.

[7] Liu, J.B.; Li, Y.L.; Li, Y.M.; Li, J.H.; Deng, Z.X. Noncovalent DNA Decorations of Graphene Oxide and Reduced Graphene Oxide Towards Water-soluble Metal-carbon Hybrid Nanostructures via Self-assembly. J. Mater. Chem. 2010, 20, 900–906.

[8] Stankovich, S.; Piner, R.D.; Chen, X.; Wu, N.; Nguyen, S.T.; Ruoff, R.S. Stable Aqueous Dispersions of Graphitic Nanoplatelets via the Reduction of Exfoliated Graphite Oxide in the Presence of Poly (Sodium 4-Styrenesulfonate). J. Mater. Chem. 2006, 16, 155–158.

[9] Stankovich, S.; Dikin, D.A.; Piner, R.D.; Kohlhaas, K.A.; Kleinhammes, A.; Jia, Y.; Wu, Y.; Nguyen, S.T.; Ruoff, R.S. Synthesis of Graphene-based Nanosheets via Chemical Reduction of Exfoliated Graphite Oxide. Carbon 2007, 45, 1558–1565.

[10] Park, S.; Ruoff, R.S. Chemical Methods for the Production of Graphenes. Nat. Nanotechnol. 2009, 4, 217–224.

[11] Zhang, J.; Yang, H.; Shen, G.; Cheng, P.; Zhang, J.; Guo, S. Reduction of graphene Oxide via L-ascorbic Acid. Chem. Commun. 2010, 46, 1112–1114.

[12] Gao, J.; Liu, F.; Liu, Y.; Ma, N.; Wang, Z.; Zhang, X. Environment-friendly Method to Produce Graphene that Employs Vitamin C and Amino Acid. Chem. Mater. 2010, 22, 2213–2218.

[13] Fernandez-Merino, M.J.; Guardia, L.; Paredes, J.J.; Rodil, S.V.; Fernandez, P.S.; Alonso, A.M. Vitamin C is an Ideal Substitute for Hydrazine in the Reduction of Graphene Oxide Suspensions. J. Phys. Chem. C 2010, 114, 6426–6432.

[14] Zhu, C.; Guo, S.; Fang, Y.; Dong, S. Reducing Sugar: New Functional Molecules for the Green Synthesis of Graphene Nanosheets. ACS Nano 2010, 4, 2429–2437.

[15] Akhavan, O.; Ghaderi, E. Escherichia coli Bacteria Reduce Graphene Oxide to Bactericidal Graphene in a Self-Limiting Manner. Carbon 2012, 50, 1853–1860.

[16] Gurunathan, S.; Han, J.W.; Epakayala, V.; Jin-Hoi, K. Microbial Reduction of Graphene Oxide by Escherichia Coli: A Green Chemistry Approach. Colloids Surf. B 2013, 102, 772–777.

[17] Wang, G.; Qian, F.; Saltikov, C.W.; Jiao, Y.; Li, Y. Microbial Reduction of Graphene Oxide by Shewanella. Nano Res. 2011, 4, 563–570.

[18] Mhamane, D.; Ramadan, W.; Fawzy, M.; Rana, A.; Dubey, M.; Rode, C.; Lefez, B.; Hannoyer, B.; Ogale, S. From Graphite Oxide to Highly Water Dispersible Functionalized Graphene by Single Step Extract-induced Deoxygenation. Green Chem. 2011, 13, 1990–1996.

[19] Thakur, S.; Karak, N. Green Reduction of Graphene Oxide by Aqueous Phytoextracts. Carbon 2012, 50, 5331–5339.

[20] Wang, Y.; Shi, Z.X.; Yin, J. Facile Synthesis of Soluble Graphene via a Green Reduction of Graphene Oxide in Tea Solution and its Biocomposites. ACS Appl. Mater. Interfaces 2011, 3, 1127–1133.

[21] Hlady, V.; Buijs, J. Protein Adsorption on Solid Surfaces. Curr. Opin. Biotechnol. 1996, 7, 72–77.

[22] Nakaniishi, K.; Sakiyama, T.; Imamura, K. On the Adsorption of Proteins on Solid Surfaces, a Common but Very Complicated Phenomenon. J. Biosci. Bioeng. 2001, 91, 233–244.
[23] Liu, J.; Fu, S.; Yuan, B.; Li, Y.; Deng, Z. Toward a Universal “Adhesive Nanosheet” for the Assembly of Multiple Nanoparticles Based on a Protein-induced Reduction/Decoration of Graphene Oxide. J. Am. Chem. Soc. 2010, 132, 7279–7281.

[24] Nisbet, A.D.; Saundry, R.; Moir, A.; Fothergill, L.A.; Fothergill, J.E. The Complete Amino-acid Sequence of Hen Ovalbumin. Eur. J. Biochem. 1981, 115, 335–345.

[25] Laemmli, U.K. Cleavage of Structural Proteins During the Assembly of the Head of Bacteriophage T4. Nature 1970, 227, 680–685.

[26] Luo, Z.; Lu, Y.; Somers, L.A.; Johnson, A.T.C. High Yield Preparation of Macroscopic Graphene Oxide Membranes. J. Am. Chem. Soc. 2009, 131, 898–899.

[27] Paredes, J.J.; Rodil, S.V.; Alonso, A.M.; Tascon, J.M.D. Graphene Oxide Dispersions in Organic Solvents. Langmuir 2008, 24, 10560–10564.

[28] Niyogi, S.; Bekyarova, E.; Itkis, M.E.; Zhang, H.; Shepperd, K.; Hicks, J.; Sprinkle, M.; Berger, C.; Lau, C.N.; deHeer, W.A.; Conrad, E.H.; Haddon, R.C. Nano Lett. 2010, 10, 4061–4066.

[29] Cui, P.; Lee, J.; Hwang, E.; Lee, H. One-pot Reduction of Graphene Oxide at Subzero Temperatures. Chem. Commun. 2011, 47, 12370–12372.

[30] Partha, K.T.; Kuila, T.; Kim, N.; Bae, S.H.; Yu, D.S.; Lee, J.H. Simultaneous Bio-functionalization and Reduction of Graphene Oxide by Baker’s Yeast. Chem. Eng. J. 2012, 183, 526–533.

[31] Kuila, T.; Bose, S.; Khanra, P.; Mishra, A.K.; Kim, N.H.; Lee, J.H. A Green Approach for the Reduction of Graphene Oxide by Wild Carrot Root. Carbon 2010, 50, 914–921.

[32] Jha, S.K.; Roth, M.; Todde, G.; Buchanan, J.P.; Moser, R.D.; Shukla, M.K.; Subramanian, G. First-principles Study of the Interactions between Graphene Oxide and Amine-functionalized Carbon Nanotube. J. Phys. Chem. C 2018, 122 (2), 1288–1298.

[33] Wu, X.; Hu, Y.; Jin, J.; Zhou, N.; Wu, P.; Zhang, H.; Cai, C. Electrochemical Approach for Detection of Extracellular Oxygen Released From Erythrocytes Based on Graphene Film Integrated with Laccase and 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic Acid. Anal. Chem. 2010, 82, 3588–3596.

[34] Krimm, S.; Bandekar, J. Vibrational Spectroscopy and Conformation of Peptides, Ploypeptides, and Proteins. Adv. Protein Chem. 1986, 38, 181–364.

[35] Shao, Q.; Wu, P.; Xu, X.; Zhang, H.; Cai, C. Insight Into the Effects of Graphene Oxide Sheets on the Conformation Andactivity of Glucose Oxidase: Towards Developing a Nanomaterial-Basedprotein Conformation Assay. Phys. Chem. Chem. Phys. 2012, 14, 9076–9085.

[36] Lee, D.Y.; Khatun, Z.; Lee, J.H.; Lee, Y.K.; In, I. Blood Compatible Graphene/Heparin Conjugate Through Noncovalent Chemistry. Biomacromolecules 2011, 12, 336–341.

[37] Xie, J.P.; Zheng, Y.G.; Ying, J.Y. Protein-directed Synthesis of Highly Fluorescent Gold Nanoclusters. J. Am. Chem. Soc. 2009, 131, 888–889.

[38] Zhang, Y.; Zhang, J.; Huang, X.; Zhou, X.; Wu, H.; Guo, S. Assembly of Graphene Oxide-enzyme Conjugates Through Hydrophobic Interaction. Small 2012, 8, 154–159.