Recent Advances on Raman Spectroscopy of Graphene: Towards Biosensing Applications

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Abstract: Graphene displays extraordinary electronic, optical, and mechanical properties, generating substantial scientific interest and presenting vast potential across various applications. Raman spectroscopy is a versatile tool for identifying and characterizing the chemical and physical properties of newly discovered carbon-based materials like graphene, carbon nanotubes, and their artificial lattices. The Raman-active vibrational modes of graphene-related materials are sensitive to structural, electrical, and interfacial modifications. This sensitivity allows Raman spectroscopy to effectively study the interaction between graphene and other materials, such as molecules and bio-related samples, towards biosensing applications. This review summarizes the recent advances in Raman spectroscopy applied to graphene and its related materials, encompassing perspectives from fundamental research to practical applications, especially bio-related applications.

Keywords: Graphene, Phonons, Raman spectroscopy, Biosensing, Molecular Interaction

1 Introduction

Since its discovery in 2004[1], graphene, a single layer of carbon atoms organized in a hexagonal lattice, has been a game-changer in materials science. It exhibits exceptional characteristics such as near-ballistic carrier mobility, high thermal conductivity, and distinctive optical and mechanical properties.[2-4] These qualities position it as a highly promising material for a wide range of applications. These include nanoelectronics, nanomechanics, batteries, and sensors, as well as flexible and printable optoelectronic and photonic devices[5-7].

Raman spectroscopy is a powerful, non-destructive, and high-resolution technique that provides insight into the vibrational, rotational, and other low-frequency modes of materials, offering valuable information about molecular structures, crystal symmetries, and electron-phonon interactions. Key features in the Raman spectra of graphene include the G band (related to in-plane vibrations), the 2D band (providing information on the number of graphene layers and their stacking), and the D band (indicating the presence of defects). Although Raman spectra of different graphene-related materials exhibit a few distinct, prominent characteristics, these bands' position, line width, and intensity provide relevant information about the structures and electronic properties. Furthermore, when subject to external influences like defects, doping, strain, magnetic fields, and temperature variations, the electronic and lattice vibration properties undergo substantial changes, which can be identified by Raman spectroscopy. By analyzing the Raman spectra of intrinsic single-layer graphene (SLG) and few-layer graphene (FLG) alongside their reactions to these external perturbations, Raman spectroscopy has found extensive applications in probing the characteristics of graphene materials and understanding their impact on the functionality of associated devices. This review provides an overview of the basics and recent advances in Raman study of graphene and its artificial structures and the emerging application in Raman enhancement and bio-related sensing.

2 Graphene, graphene derivatives, and artificial structures

2.1 Raman spectroscopy of graphene

SLG refers to a single layer of carbon atoms arranged in a honeycomb structure, where each carbon atom is covalently bonded to three neighboring carbon atoms. As shown in Figure 1a, the unit cell of the crystal lattice in SLG consists of two carbon sites, A and B. In few-layer graphene (FLG), individual graphene layers stack in AB stacking order, in which the adjacent layers have a relative rotation of 60° in the out-of-plane direction (c-axis), as shown in Figure 2b. The interlayer spacing is typically measured to be 0.35 nm.

To interpret the Raman spectra of graphene, the phonon dispersions of SLG consist of three acoustic branches (A) and three optical branches (O). The out-of-plane (Z) motion modes are significantly softer compared to the in-plane longitudinal (L) and transverse (T) modes. SLG and graphite have two and four atoms in the unit cell, respectively resulting in six and twelve phonon modes at the G point. The lattice vibration of SLG and graphite at G point can be expressed as $A_{2u} + B_{2g} + E_{1u} + E_2$ and $2(A_{2u} + B_{2g} + E_{1u} + E_2)$ respectively.[7,8] A_{2u} mode and the doubly degenerate E_{1u} mode correspond to the three acoustic modes in both SLG and graphite. Whereas three optical modes in SLG contain a doubly degenerate in-plane E_{2g} mode along with one out-of-plane mode B_{2g}. [9]

The Raman spectrum of pristine SLG contains a number of unique bands in the spectral region from 1500–3400 cm^{-1} (Figure 1d).[3,10] Figure 1c depicts the optical phonon dispersions of SLG that are important for understanding the Raman spectra.[11] The peak at ~1582 cm^{-1} corresponds to the high-frequency E_{2g} phonon at Γ.[12] Moreover, the 2D band is strongly dispersive with excitation energy because of a Kohn anomaly at the K point. The corresponding fundamental modes of the 2D and 2D′ bands, i.e., D and D′ band (Fig. 1c), require defect to be activated in the double resonance Raman scattering (DRRS); thus, they are absent in the Raman spectrum of pristine SLG.[12-14] The Raman spectra of graphene and few-layer graphene (FLG) provide crucial information about the structure and disorder information. As the number of graphene layers increases, two extra Raman modes start to evolve. Those unique modes are determined as shear and layer-breathing modes originating from the relative interlayer movements of adjacent layers. The interlayer breathing vibration mode normal to the basal plane appears at ~90 cm^{-1} and is often referred to as LB mode.[15-17] Whereas, the shear mode (at ~31 cm^{-1}) in multilayer graphene was first observed by Tan et al.[18] It provides a direct measurement of the interlayer coupling in the system.

Moreover, because of the strong correlation with the in-plane G and 2D band, the number of layers can be precisely determined using the frequency of these modes.[19-21] As the number of layers (N) increases, the intensity and the linewidth of the 2D band change due to the alteration of the electronic band (Figure 1e).[22] In addition to the layer number, this evolution of the band profile provides useful information to determine the relative orientation of the layers.[23]

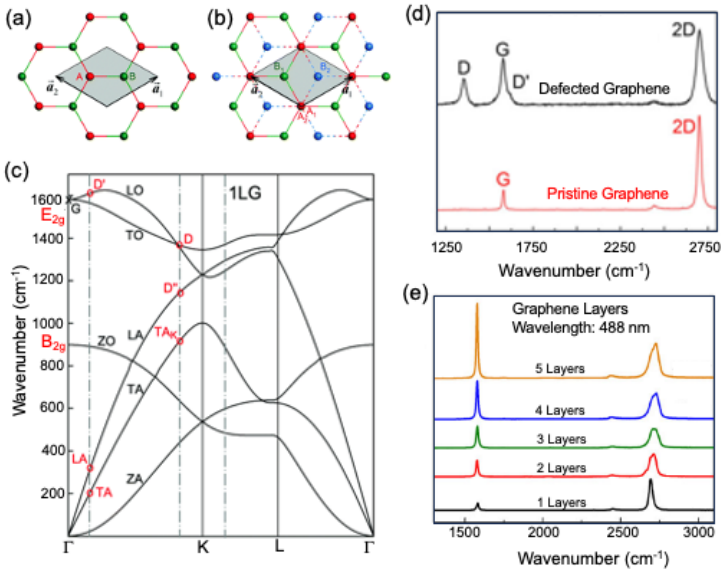

Figure 1. Phonon modes of SLG and FLG. (a) The top view of the unit cell of SLG crystal lattice shows two inequivalent carbon atoms A and B as well as unit vectors $\overrightarrow{a_1}$ and $\overrightarrow{a_2}$. **(b)** A top view of AB-stacked double layer graphene. Adapted with permission from reference[2]. ©2009, American Physical Society. **(c)** Phonon dispersion curves of SLG. The assignments of each phonon branch are labeled. Reproduced with permission from reference[11]. ©2008, American Physical Society. **(d)** Raman bands of pristine and defected graphene grown by chemical vapor deposition. Reproduced with permission from reference[3] ©2017, John Wiley & Sons, Ltd. **(e)** The evolution of Raman signature obtained from mechanically exfoliated FLG with various atomic layer counts. Reproduced with permission from reference[22]. ©2009, American Institute of Physics.

2.2 Graphene oxide (GO) and reduced graphene oxide (rGO)

Graphene oxide (GO) is obtained by treating graphite with strong oxidizers and subsequent exfoliation. GOs have a broad range of properties depending on the oxidation-induced defects[24], and are promising for use as material templates and platforms for next-generation electronic devices. However, the oxygen-containing functional groups on the GOs such as epoxy, carboxyl, and hydroxyl can hinder the controllable applications of GO-based materials[25,26]. Significant research efforts have been made to reduce GO, which is reduced graphene oxide (rGO)[27,28]. Raman spectroscopy is a versatile tool to characterize GOs and rGOs[18,29]. GO can be exfoliated in solutions such as water, producing colloidal suspensions of individual graphene oxide sheets. Those individual sheets can undergo further chemical functions and vacuum filtration towards novel composites and large-scale ensembles[30,31].

The Raman spectrum of GO sheet is presented in Figure 2a. Similar to graphene, the G band and 2D band are characteristic of sp^2 hybridized C-C bonds. The intense and broad D band and high $I(D)/I(G)$ ratio in GO confirms its lattice distortions and a large portion of sp^3-like defects caused by the oxidation[32]. The blueshift of the G peak also confirms the defects. The 2D band is very sensitive to the defects of graphene-based materials, meanwhile, the absence of 2D band in GO also represents the high disorder. In the rGO Raman spectrum (Figure 2b), the G band occurs at 1581 cm^{-1} and it represents the recovery of sp^2 network with a certain amount of defects. The decrease of $I(D)/I(G)$ ratio of rGO indicates the reduction process of GO may affect the structure of GO, resulting in a smaller number of structural disorders. Compared with GO, the 2D peak (~2580 cm^{-1}) is more significant in rGO, and the D + G is located at ~2910 cm^{-1}, indicating the restored graphite structure.

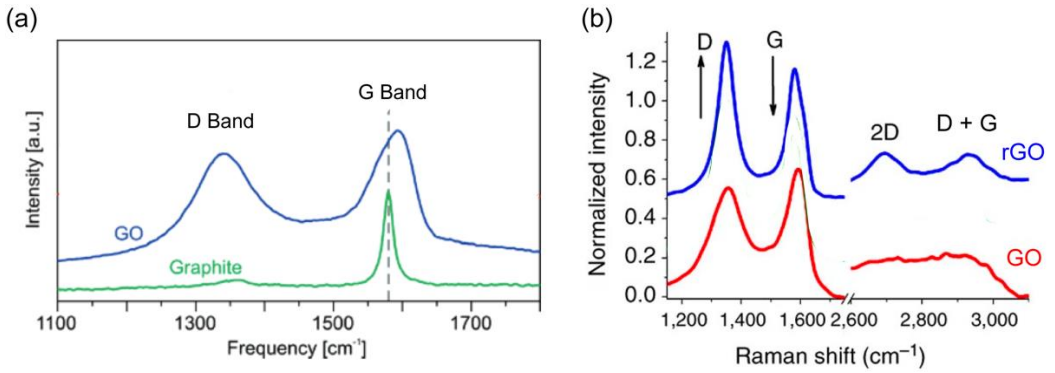

Figure 2. Raman spectra of GO and rGO. (a) Raman spectra of GO and pristine graphite. Adapted with permission from reference[32] © 2008 American Chemical Society. (b) Raman spectra of rGO and GO. Adapted with permission from reference[24] © 2010, Springer Nature Limited.

2.3 Artificial Graphene structures

Graphene flakes can be stacked into artificial structures thanks to the atomically thin van der Waals nature. *Moiré* superlattice is a fascinating phenomenon that occurs when two graphene sheets are superimposed at a slightly twisted angle. This configuration creates a larger, periodic pattern, which is a direct consequence of the interference between the two lattices shown in Figure 3a. This interference is not just a simple overlay; it gives the superlattice a unique characteristic, an angle-dependent wavevector. [26–29] This wavevector is particularly interesting because it leads to the emergence of what is known as van Hove singularities in the electronic density of states. These singularities are points in the energy spectrum of a solid where the density of available electron states experiences a sharp change, which can lead to interesting and often useful properties.

Moreover, this process also activates phonons within the interior of the graphene Brillouin zone, illustrated in Figure 3b-c. The activation of these phonons can significantly affect the material's thermal and electrical properties. In essence, forming a *moiré* superlattice through the overlaying of two graphene sheets at a small twist angle is a complex and intriguing process, with implications for the electronic and vibrational states of the material. This makes it a topic of great interest in the study and application of graphene-based materials.

While traditional Raman spectroscopy on graphene can provide insight into the fundamental properties of graphene layers, it can also be used to investigate the unique properties of the moiré superlattice in twisted bilayer graphene (TBLG). It offers information about these systems' twist angle, stacking order, and electronic band structure. One of the key features in the Raman spectra is the 2D band. The frequency and width of this band can offer valuable insights into the twist angle between the graphene layers. This is because the twist angle directly impacts the electronic properties of the TBLG (Figure 3d-e), which in turn influences the characteristics of the 2D band in the Raman spectra. In addition to the 2D band, the Raman spectra of TBLG can exhibit other features due to the presence of the *moiré* superlattice, which introduces new features in the Raman spectra that are not present in the spectra of untwisted graphene layers. The resonance effects are one of the most intriguing aspects of these additional features (Figure 3f). These effects, including single and multiple resonances and intra- and inter-valley scattering, make it possible to accurately measure the energy of superlattice-induced van Hove singularities in the electronic joint density of states.

Furthermore, these resonance effects also allow for measuring the phonon dispersion relation in TBLG. This includes the layer breathing vibrational modes, which are modes of vibration where the layers of the graphene move in a coordinated way. These modes can have important implications for the thermal properties of the material.

In summary, the Raman spectra of TBLG are a rich source of information about the material, providing insights into the twist angle, the presence of a moiré superlattice, the energy of van Hove singularities, and the phonon dispersion relation. This makes it an invaluable tool in the study and application of TBLG.[31–34]

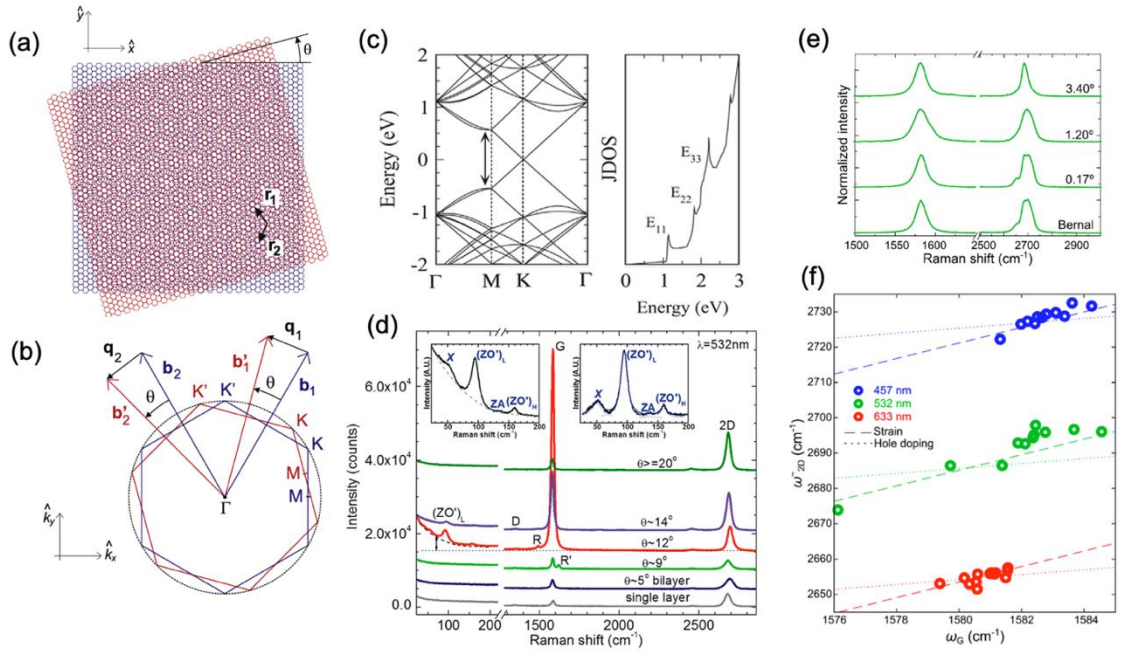

Figure 3. Raman spectra of artificially stacked graphene structure. (a) Schematics of a rotationally stacked bilayer graphene, with the red layer on top of the blue layer, generate a moire pattern. (b) The 1st Brillouin zones of the stacked layers. Adapted with permission from reference[30], © 2011 American Chemical Society. (c)The energy bands and the joint density of states (JDOS) for three van Hove singularities. (d) Raman spectra of TBLG with different angles. Adapted with permission from reference[17], © 2013 American Chemical Society (e) Raman spectra of near magic angle TBLG. Adapted with permission from reference[31], © 2022 IOP Publishing Ltd (f) Medium 2D band Raman shift (ω_{2D} ~) and G band Raman shift (ω_G) relation.

Understanding the intricate phonon behaviors and exploring the realm of artificial graphene structures has unveiled a rich landscape of possibilities in the domain of advanced materials science. The manipulation of phonon modes and the design of artificial graphene configurations have expanded our comprehension of fundamental principles and fostered the emergence of innovative applications. One important research area is an exploration of graphene's molecular interactions, where the material's remarkable sensitivity and unique molecular behavior pave the way for groundbreaking biosensing applications. For instance, graphene and its derivatives stand out in molecular interactions and biosensing due to their exceptional properties. Their high surface area is pivotal, offering extensive space for molecular interaction and adsorption, resulting in heightened sensitivity for detecting molecular changes.

Moreover, their biocompatibility allows seamless interfacing with biological molecules, cells, and tissues, making them excellent platforms for biosensing in biological systems. By easily functionalizing their

surfaces with specific molecules or functional groups, graphene enhances its selectivity and specificity in detecting target biomolecules. Furthermore, their unique optical properties, especially in Raman scattering, enable label-free and non-destructive molecular identification, a crucial aspect in biosensing without requiring additional tags or labels. For example, a recent article by Yildiz et al. has thoroughly discussed the intrinsic ripples of graphene and their effect on graphene biosensing capabilities, which is corroborated by the discussion in previous sections[33]. These characteristics enable the development of miniaturized, portable, and highly sensitive biosensors, facilitating rapid and on-site detection of biomolecules and viruses. These properties collectively position graphene and its derivatives as promising candidates for studying molecular interactions and advancing biosensing technologies, with profound implications in healthcare, environmental monitoring, and various other fields. With recent breakthroughs in studying phonon behaviors and artificial structures discussed in previous sections, our focus now shifts toward harnessing these properties to revolutionize biosensing technologies, marking a pivotal stride in utilizing graphene's exceptional characteristics for real-world applications.

3 Graphene is used as surface enhanced Raman scattering (SERS) platform

3.1 Molecular interaction mechanism

The interaction between graphene and molecules originated from its distinctive 2D structure and exceptional surface sensitivity, which grants a substantial surface area and remarkable surface sensitivity. Raman spectroscopy is a potent tool for examining these interactions due to its ability to detect minute vibrational changes at the molecular level. When molecules or atoms interface with graphene, they influence its electronic and vibrational properties, inducing changes in the Raman spectra. Alterations such as spectral shifts, intensity changes, and peak broadening observed in the Raman spectra of graphene offer valuable insights into the nature of molecular interactions, including charge transfer, chemical bonding, and structural modifications. This analytical approach enables the study of graphene's adsorption, doping, and functionalization, contributing to a deeper understanding of diverse molecular interactions on this 2D material. This comprehension holds significance for various applications such as biosensing, nanoelectronics, and catalysis, where the manipulation of graphene's properties through its interaction with molecules and atoms is pivotal. The Surface-Enhanced Raman Scattering (SERS) fundamentals of graphene interactions with molecules revolve around the unique properties of graphene that amplify Raman signals. Graphene's high surface area, electronic structure, and plasmonic behavior contribute to its SERS capabilities. When molecules adsorb onto or near graphene, the electromagnetic field enhancement in the vicinity of graphene enhances the Raman signals of these molecules[34]. Graphene's ability to confine and amplify electromagnetic fields allows for substantial signal enhancement, facilitating the detection and characterization of molecules at very low concentrations. Additionally, the two-dimensional nature of graphene enables precise control over its interaction with target molecules, leading to tailored and highly sensitive detection in SERS applications.

Both chemical and electromagnetic mechanisms work in tandem with graphene, enabling highly sensitive and specific detection in Raman spectroscopy. The chemical mechanism deals with modifications in the molecular environment induced by direct interactions with graphene. In contrast, the electromagnetic mechanism harnesses graphene's unique electromagnetic properties to amplify the Raman signals of nearby molecules. This synergy makes graphene an excellent platform for various sensing and detection applications in Raman spectroscopy.

3.2 Graphene-enhanced Raman scattering (GERS)

Graphene-enhanced Raman scattering (GERS) has been widely employed in manifold research fields and explored for detecting trace amounts of molecules. Several investigations have focused on the interactions between graphene and adsorbed molecules, allowing researchers to develop new ideas for potential applications. Typically for fluorescent dyes Raman features are buried by the intense fluorescent

background. It has been a long-term question of how to get the Raman signal of molecules from their fluorescence background. Pristine graphene has proven its ability to quench organic molecules' fluorescence background when adsorbed on the graphene surface. A quenching factor on the order of 10^3. When these dye molecules are adsorbed on graphene, considerable π-π interactions are formed. In general, interactions of π electrons, vibronic coupling, and the interplay between the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) within the Fermi level of graphene enhances the Raman signal.

Investigation of the interaction between graphene family and molecules has been a hot topic for several years. The interplay between probe molecule orientation and graphene can promote charge transfer, enhancing the interfacial dipole and polarizability. Copper phthalocyanine (CuPc), an interesting probe molecule has macrocycle and isoindole ring-related vibrations which enhance the π–π interactions between CuPc and graphene. It has been found that different molecular orientations of this probe molecule can activate superior properties which is beneficial in sensing applications[21,35,36]. When CuPc changes its orientation from upstanding to lying down configuration, the molecule's dipole moment effectively shifts the energy levels of graphene and results in an enhanced Raman signal[37]. Tricyanofuran group (TCF), a high dipole moment probe molecular entity can facilitate an enhanced Raman signal due to strong interfacial coupling[38]. Zhang and coworkers studied graphene-thickness-dependent GERS in detail using the probe molecule protoporphyrin IX (PPP)[36]. The results concluded that there is an abnormal graphene-thickness dependence of GERS between monolayer and bilayer graphene. Probe molecule attachment using vacuum thermal deposition resulted in similar GERS enhancement for all graphene layers (1-6 layers).
On the other hand, adsorbing probe molecules by solution soaking results in different enhancement factors for monolayer and bilayer graphene. Ling et al. investigated the first-layer effect of graphene, providing solid evidence for chemical enhancement of GERS[39]. Briefly, using Langmuir-Blodgett technique (LB), PPP with different layer numbers of the LB film on graphene was fabricated and corresponding Raman measurements were acquired. It has been found that Raman signal from the first monolayer LB film of PPP has a larger contribution to the Raman enhancement than that from subsequent monolayers. Additionally, it has been found that Raman signal enhancement depends on the interaction of PPP's hydrophilic and hydrophobic anchoring groups in contact with graphene. This illustrates there is distance dependence of the charge transfer on Raman enhancement. For a symmetric molecule like CuPc, such a difference in Raman signal was not observed based on how CuPc was deposited (CuPc on top of graphene or CuPc on bottom of graphene). Moreover, the effect of graphene substrate functionalization and excitation wavelength was also explored in the literature, providing insight into graphene-based molecular sensing[40,41].

3.3 Enhancement of GERS by integration with plasmonic nanostructures

The hybrid is expected to outperform individual counterparts when graphene is integrated with plasmonic nanostructures. By depositing SERS-active metallic nanoparticles over a flat graphene surface, researchers achieved a new kind of SERS substrate referred to as a graphene-mediated SERS (G-SERS) substrate, demonstrating additional advantages over conventional SERS substrates. In these graphene/metal hybrid structures, plasmonic hot spots spread throughout an atomically flat surface are responsible for Raman enhancement. Schedin et al. have shown the capacity of graphene to probe the SERS activity[42]. In this work, the researchers used varying-sized gold nanoparticle arrays to demonstrate SERS activity. They found significant enhancement of the signal at 633 nm. The results are also corroborated with theoretical simulations.

Moreover, thickness-dependent and position-sensitive SERS activity was explored by other research groups[43,44]. For instance, G-SERS substrate made out of monolayer graphene with gold/silver nanoislands has provided a way for flat SERS platform. Here, the graphene/metal hybrid provides a more homogeneous molecular orientation for the probe molecules. Additionally, this platform enhances spectral reproducibility and suppresses broad background peaks. More importantly, the developed substrate allows physical separation of the analyte and Au by graphene, which reduces photo-induced damage to the analyte. The

enhancement of the Raman signal, up to $2x10^5$ times, occurs when single-layer graphene (SLG) is deposited onto a nanohole array covered with silver[45]. The SLG is placed on top of an array of silver-covered nanoholes in a polymer and is covered with water. This enhancement arises from an increase in the confinement of the electromagnetic field at the SLG location, which leads to amplified light absorption within the graphene specifically at the excitation wavelength. Li et al. engineered a GO/AgNPs/Cu film (GO/Ag/Cu) hybrid on a silicon pyramid array[46]. Leveraging the robust electric field enhancement capability of pyramid Cu films and AgNPs, this hybrid construct showcased exceptional sensitivity, detecting concentrations as low as 10^{-15} M of R6G. The SERS signals exhibited remarkable attributes, including high sensitivity, uniformity, and stability when R6G was employed as the probe molecule. Simulation findings further underscored that the electric field enhancement formed between the AgNPs and extended between the AgNPs and the Cu film.

Due to interlayer coupling, 2D heterostructure stacking can significantly enhance Raman scattering compared to the individual layers. In this regard, graphene-based 2D van der Walls heterostructures were developed with the aim of ultrasensitive molecular detection. Seo et al. demonstrate a graphene/ReO_xS_y vertical heterostructure as an ultrasensitive SERS platform with synergistic effects of complementary resonances and dipole-dipole interactions[47]. The heterostructure showed higher enhancement for R6G than ReO_xS_y and graphene as well as ultralow limit of detection (LOD) of 10^{-15} M and a perfect linear relation between the Raman intensity and R6G concentration. The improved LOD is attributed to enhanced electron transfer and exciton resonance. Ghopry et al. developed a high-performance SERS substrate in heterostructure configuration using MoS_2 and WS_2 with graphene[48]. Due to strong dipole-dipole interaction at the heterostructure interface, they were able to achieve extraordinary sensitivity of 10^{-12} M with R6G probe molecule. The same authors devised a novel method to boost the CM effect on graphene SERS substrates, involving two key components: short-period UVC irradiation of graphene and atomic layer deposition to decorate Pt-NPs on the graphene surface[49]. The former yielded a remarkable 270% enhancement, attributed to the activation of the graphene surface and its p-doping, enhancing the attachment of R6G molecules and facilitating charge transfer. This was achieved by altering the graphene surface from hydrophobic to hydrophilic and shifting the Fermi energy (p-doping) post-UVC exposure. Introducing Pt-NPs further enhanced this by 250%, leading to additional p-doping of graphene. This shift in the graphene's Fermi energy facilitated charge (hole) transfer at the R6G/graphene interface. Li and colleagues introduced a novel approach to produce a highly sensitive and mechanically robust flexible SERS substrate using Ti3C2Tx MXene@graphene oxide/Au nanoclusters (MG/AuNCs) through wet spinning and subsequent in situ reduction processes[50]. This hybrid structure showcases remarkable attributes including a low detection limit of 1×10^{-11} M, an enhancement factor of 2.01×10^9, excellent signal repeatability (RSD = 9.80%), and long-term stability (75% signal retention after 90 days of storage) when detecting R6G molecules.

3.4 SERS manipulation

Doping is an effective approach to manipulating electronic, chemical, and optical properties of a range of materials. Researchers have attempted to incorporate dopants into graphene to achieve enhanced physico-chemical properties. Terrones and coworkers reported that the introduction of different dopant atoms has the potential to significantly alter its local electronic and chemical properties[51]. One report reported the successful synthesis of nitrogen-doped graphene (NG) via the atmospheric-pressure chemical vapor deposition (AP-CVD) method. They demonstrated that the introduction of nitrogen atoms within the same graphene sub-lattice could significantly change its electronic and chemical properties[52]. Results showed the double substitution of N dopants that existed in the same graphene sublattice.

Additionally, RhB dye was utilized to illustrate outstanding Raman enhancement of NG. In a follow-up study, the authors reported the GERS effect of NG[53]. The results revealed that NG has a higher density of states (DOS) and more appropriate energy level spacing than primary graphene (PG), which promotes the charge transfer resonance between the substrates and the probe molecules. As a result, the detection limit

of RhB on NG can be reduced to 5×10^{-11} M. The authors also demonstrated that Si-doping could favor enhanced adsorption of different molecules onto graphene, leading to a significantly improved GERS effect. Theoretical simulations demonstrated that the introduction of silicon induces local curvature around the substitutional site, which enhances the interaction of dyes with graphene.

Due to special structural configuration, vertically oriented graphene (VG) has been explored as a better fluorescence quencher. For example, VG is characterized by a large surface area, high optical absorption, mechanical stability, and chemical stability. Cai and coworkers reported that the quenching efficiency of VG is more than three times that of graphene[52,54]. By utilizing aromatic dyes, such as eosin Y, rhodamine B, methylene blue, and gallocyanine they found that better fluorescence quenching is attributed to energy and electron transfer between dyes and VG. The polarized excitation and detection of Raman scattering for pentacene molecules grown on graphene substrates was recently used to determine the orientation of pentacene. The results imply that pentacene and graphene exhibit several preferred angles due to atomic interactions[54]. Berrellez-Reyes et al. reported the interaction of a graphene oxide (GO) substrate and a fluorescent organic molecule rhodamine B (RhB) studied by Raman spectral deconvoluted scanning[55]. They were able to achieve spectral deconvolution of a larger number of spectra with overlapping bands of GO and RhB.

A recent study introduced a method enabling the active manipulation of SERS signals through photoinduced processes[56]. This technique leverages UV irradiation to dynamically adjust the Fermi level of graphene, influenced by the adsorption and desorption of gas molecules. The effectiveness of this method was confirmed across various gas atmospheres including O_2, CO_2, N_2, and air. Moreover, its versatility was demonstrated with different analytes. The SERS substrate utilized in this approach comprises an Ag-coated polystyrene (PS@Ag) nanoarray covered by a self-assembled reduced graphene oxide (rGO) film. The modulation of the SERS signal is attributed to the alteration of the rGO's Fermi level induced by UV-driven adsorption and desorption of gas molecules, thereby affecting the charge transfer between the substrate and analytes. Significant progress has been made in using graphene-based substrates for molecular sensing and potential transformation into practical applications. Due to an atomically thin layer and chemically inert surface, graphene provides a pathway to sense molecules in a uniform, repeatable, and quantifiable manner. Several phenomena related to graphene-based molecular sensing have been investigated over the past several years. Several factors need to be considered when developing a graphene-based sensing platform to achieve the best use of the charge transfer mechanism. Orientation/polarity of probe molecule, effect of dipole moment of probe molecule, distribution of probe molecule, first layer effect, layer (thickness) effect, impact of laser wavelength, and functionalization of the substrate are the criteria when developing suitable substrate[57]. These processes are depicted in Figure 4. In-depth experimental and theoretical investigations are required to better understand underlying effects. Bringing this star material into practical applications requires a deep understanding of the graphene family materials and molecules.

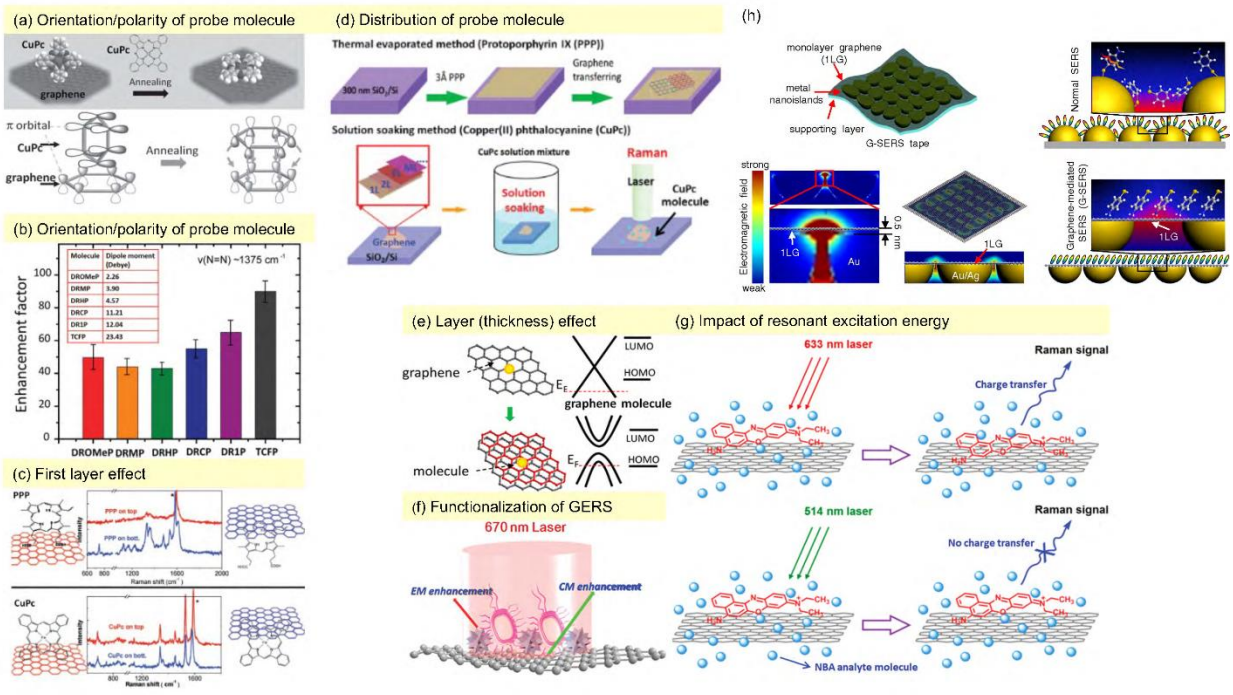

Figure 4. Molecular interaction mechanism– (a)-(g) Characteristics of GERS effect. Adapted with permission from Reference[58] © 2019 Wiley Online Library. (h) Illustration of fabrications and measurements of graphene-based SERS substrate. Adapted with permission from Reference[44] © 2012 *Proceedings of the National Academy of Sciences*.

4 Towards Graphene-Based Biosensing

Recent years have witnessed a boom in biomedical research based on 2D materials, which is rapidly evolving to broaden the spectrum of emerging 2D materials[59]. Driven by increasing interest in personalized medicine, several biosensing platforms based on 2D materials have been proposed and demonstrated. Graphene represents potentially transformative structure within the 2D material family characterized by unprecedented optical and electronic properties. The essence of the biological applications of graphene and its family members relies on molecular interactions between molecules and graphene surface. These interactions are dictated by properties such as surface energy, surface charge, and hydrophobicity. Typical graphene-based materials are sensitive to a range of incitements such as biomolecules, optical excitation, and electrical fields. Out of them graphene biomolecule interactions based on surface-enhanced Raman spectroscopy (SERS) have been explored extensively owing to applications in biosensing[40,44]. Graphene and its derivatives, such as graphene oxide (GO) and reduced graphene oxide (rGO), are also promising in many biosensing applications. This applicability mainly arises from large specific surface areas, fluorescence quenching capability, high electrical and thermal conductivities, and excellent mechanical properties. This section summarizes recent advances in graphene biomolecule interactions based on SERS.

Surface-sensitive techniques have been utilized to characterize graphene-molecule interactions. Raman spectroscopy is a well-established optical technique that has emerged as a powerful analytical tool in biosensing applications due to its label-free, multiplex, and non-destructive nature[60,61]. SERS is an optical detection method based on electromagnetic enhancement in the field that has great advantages in characterizing how molecules interact with graphene and its derivatives. As widely accepted, SERS enhancement mainly depends on the substrate type (metallic or semiconductor-based). Hot spots originated from localized surface plasmon resonance for metallic substrates, caused by collective oscillation of free electrons[62,63]. On the other hand, for semiconductor-based substrates, SERS enhancement is dictated by

charge transfer processes that take place between substrate and chemisorbed molecules. The SERS effect of graphene was first discovered by Ling et al., who demonstrated that probe molecules placed on graphene surfaces have much higher Raman signals than when they are deposited on bare substrates[64]. Additionally, this represents an alternative to SERS as graphene serves as a surface that can produce reliable Raman spectra. The evolution of utilizing graphene as a potential SERS substrate broadens the horizons of SERS research and transforms it into practical biosensing applications.

Biosensing is an important subfield of sensing that has been commonly employed for identifying and quantifying biological analytes. Generally, biosensing adopts different mechanisms, such as optical, thermal, electrochemical, and electrical. Graphene and its family members have been widely explored to accurately detect biomolecules and biomarker screening for clinical diagnosis, treatments, and biosensing applications. Enhancing the Raman signal is paramount when detecting trace levels of molecules. In this regard, SERS attracted significant research interest as it enables single-molecule detection and due to other merits. One of the barriers to traditional noble metal SERS detection is the nonuniform distribution of hot spots, which complicates detection schemes. The idea of exploiting graphene to overcome limitations in SERS has attracted growing interest in the past decade. Recently reported graphene-enhanced Raman scattering (GERS) has revolutionized the interest in the use of SERS in biosensing applications. The origin of GERS is believed to be chemical mechanism (CM) and can induce a synergistic effect with an electromagnetic mechanism (EM). GERS is particularly useful for detecting biological processes, disease stages and progression, as well as detection of biomarkers. For instance, Huang et al. proposed a reproducible strategy to differentiate the SERS signal of blood constituent proteins[65]. Researchers could identify Raman fingerprints of biomacromolecules, hemoglobin, and albumin using graphene as a substrate. Importantly, in the absence of graphene, the Raman signal of albumin with low concentration and low irradiance is not detectable while Raman modes are greatly enhanced when in contact with graphene. Furthermore, results revealed important properties of biomolecules, such as oxidation state, structure, and environment. The results indicated that the origin of the enhancement is based on π-π interaction between graphene and biomolecules.

Graphene-assisted Raman spectroscopy is capable of distinguishing Alzheimer disease (AD) brain tissues and healthy control brain samples. For instance, Wang et al. demonstrated machine learning and graphene-assisted Raman spectroscopy technique for rapid biomarker screening of AD[66]. The experiment first collected Raman spectra from healthy and AD mice followed by machine learning classification. By contacting monolayer graphene with brain tissues, the accuracy was increased from 77 to 98 % in machine learning classification. In this study, graphene helps to improve the signal-to-noise ratio due to higher thermal conductivity and fluorescence quenching capability. Figure 5 shows the overall workflow of the analysis. This study illustrates that Raman spectroscopy has significant implications for early disease diagnosis. Demeritte et al. established a GO-based Raman biosensor platform for selective separation and label-free identification of AD biomarkers from whole blood[40]. In this study β-amyloid as an AD biomarker was separated and detected using a portable SERS probe with a detection limit of 100 fg/mL. Here GO serves not only as a template for nanoparticles but also as a Raman signal enhancer. Another study looked at the fundamental characteristics of graphene-based hybrid material structures that are essential for sensing through noncovalent interactions[67]. Here, the researchers reported a new development of GERS called double-sided GERS due to graphene interacting with molecules on both sides and providing separate enhancements.

Briefly, silicon wafer was covered by 100 nm thick Ni film. Then, a layered structure on top of Ni was constructed, composed of human kidney tissue, single-layer graphene, and biocompatible nitrocellulose molecules (Mo/Gr/Ki/Ni). Enhancement factor values varying from 2.1 to 10.1 were observed under different conditions explained by molecule-graphene coupling through noncovalent interactions. In-depth knowledge of the GERS is provided by this work, which opens a new avenue in engineering graphene-based drug delivery systems, imaging, and sensing devices. GERS and drop-coating deposition Raman

(DCDR) spectroscopy recently allowed for a new analytical hybrid platform for detecting human interleukin-6 (IL-6), a key cytokine that plays an important role in the immune system and inflammatory response[68]. This study reported detection limit of 1pg/mL in PBS. In a parallel work the same research group utilized the above concept to investigate different antigen–antibody interactions and their surface distribution[69]. They used monoclonal antibodies of interleukin-6 (mabIL-6) onto amine functionalized graphene substrate. The authors mentioned that the mabIL-6 coffee ring is where the antigen/antibody selective interaction occurs.

Additionally the above technique has proven its ability for amino acid analysis with detection limits in tens of ng/mL[70]. Graphene is capable of wrapping beads, pollen grains, and live cells like skin. Xu et al. capitalized on those properties and developed hybrid graphene-polymer-based self-folding flexible skin-like SERS substrate that can be applied for spatial mapping of MDA-MB-231 breast cancer cells[71]. They measured Raman maps at different layers of cells and built a 3D map of cells to obtain information on spectral markers.

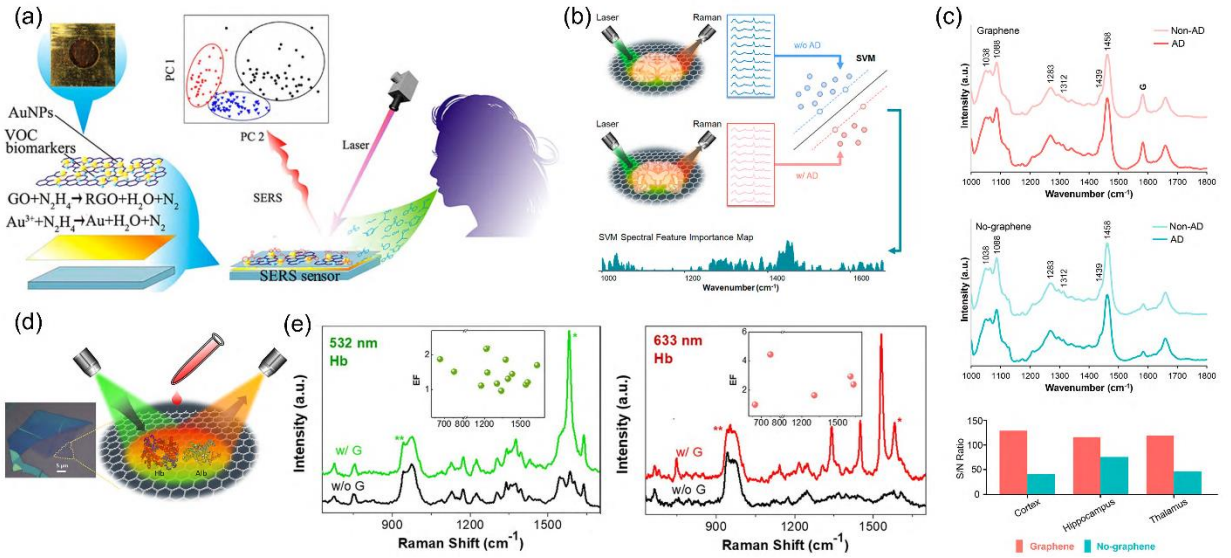

Figure 5. Biosensing application. (a) Schematic diagrams of SERS sensor and overview of the processes involved in the breath. Adapted with permission from Reference[72] © 2018 American Chemical Society. (b) Workflow of graphene-assisted Raman signals' data collection, preprocessing, and machine learning classification and interpretation. (c) Preprocessed Raman spectra in the cortex region with and without AD, for the case of in the presence and absence of graphene and S/N of every brain region, measured with and without graphene. Adapted with permission from Reference[66] © 2018 American Chemical Society. (d) Optical microscopic image of a graphene flake with illustration of the GERS measurement (e) GERS measurement of old constitute proteins. Adapted with permission from Reference[67]© 2018 American Chemical Society

Identifying tumor biomarkers is an important step in determining if cancer is present and its origin. Recently, Jiang and coworkers developed a GO-based ultrasensitive SERS substrate for the detection of carcinoembryonic antigen (CEA), one of the most common tumor biomarkers[73]. By integrating gold nanorods with GO, this SERS platform provided detection limit of 3.01 pg mL−1 for CEA detection. Cui et al. successfully monitored 14 volatile organic compounds (VOC) biomarkers in exhaled gas to

distinguish early and advanced gastric cancer patients from healthy individuals[74]. The developed sensor can enrich biomarkers from breath due to the presence of GO on the Au substrate. Various machine learning algorithms have been used to process SERS data sets. The healthy, early, and advanced samples clustered into separate groups. This strategy has been successfully used to distinguish simulated breath samples and patients' breath samples with a sensitivity higher than 83% and a specificity of more than 92%, showing great potential for clinical transformation. The capability of SERS detection of adenosine in human urine samples based on graphene/copper nanoparticle hybrid (G/CuNP) was also reported[72]. This hybrid substrate showed a remarkable SERS response for adenosine (detected concentration in serum ~5 nM). It has been found that the graphene shell can enrich and fix the adenosine molecules. Ma et al. developed GO-wrapped gold nanoparticles for intracellular Raman imaging of HeLa cancer cells[75]. Due to SERS enhancement provided by the gold core and signature Raman features of GO shell, this hybrid material is promising in next generation of nanotherapeutics. Jiang et al. presented a novel label-free approach for detecting living K562 chronic myeloid leukemia cells. They utilized a hybrid graphene/gold nanopyramid-based surface-enhanced Raman scattering (SERS) method via a grid structure[76]. The graphene sheet enabled the identification of SERS hot spots and offered chemical enhancement for the biological constituents under investigation.

Ultrasensitive and rapid detection of DNA is very important for a wide range of fields. Duan et al. developed a sandwich structure based on thiolated graphene oxide (tGO) nanosheets and gold@silver nanoparticle arrays[77]. As graphene acts as an atomically thin separator between plasmonic nanoparticles, the sandwich structure generates a strong, controllable electromagnetic field, and SERS signal inside the gap. tGO anchored Raman active molecule conjugated DNA into the nanogaps hot spots between nanoparticle layers via π-π interaction. Additionally, in the presence of DNA, nanoparticles form agglomerates resulting in increased intensity of SERS signal. The work of Gupta and coworkers configured an enzymatic sense glucose biosensor platform based on Ag@Au nanoparticles modified graphene oxide nanocomposite[78]. In their work, biosensor was fabricated by loading glucose oxidase (GOD) onto mercaptophenyl boronic acid (MBA) terminated nanocomposite. The Raman peaks of MBA decreased with the addition of glucose varying from 2-6 mM.

Additionally, MBA contributed to sensitivity and selectivity through complexation between the glucose's boronic acid and diol groups. In another work, Chattopadhyay et al. developed a non-enzymatic glucose sensing protocol by SERS on CVD graphene[79]. The intensity ratio of the 1122 cm^{-1} peak of glucose to the 2D peak of graphene varied linearly with glucose concentration providing a method for the detection of glucose. Results were explained based on charge transfer between glucose and graphene. Li et al. utilized the SERS activity of oxidized ascorbic acid in the GO and gold nanorod hybrid substrate to sensitively detect glucose[80]. The sensing mechanism has been attributed to the effect of density and viscosity of liquid glucose on the SERS activity of oxidized ascorbic acid. A worth-mentioning study that was recently published used malachite green (MG) to label dopamine (DA), one of the most common neurotransmitters that can characterize the differentiation of neural stem cells[81]. Briefly, the combination of tooth-like gold nanoarrays and GO nanosheets enhances the Raman signal intensity uniformly. The aptamers used in the study bound to the GO surface through π-π interactions. The strategy to detect DA released from single cells is as follows. First, the aptamers begin to bind to DA and the conformation of the nucleic acid aptamers changes. This results in the release of DA from GO. The MG labeled aptamers are thus separated from the SEERS substrate. As a result, the degree of differentiation of the neural stem cells can be evaluated from the decreased SERS signal. With the developed approach, in situ detection of DA was possible in a wide range of concentrations, 10^{-4} to 10^{-9} M.

Recent studies have highlighted graphene's role as an ultra-thin separator amid plasmonic nanoparticles (NPs), establishing an exceedingly narrow plasmonic gap[82]. In this hybrid system, rGO was positioned between Ag and Au nanostructures, creating an Ag-RGO-Au system with a robust chemical mechanism, primarily driven by charge transfer between RGO and the target molecules. Evaluating the SERS sensor built on the Ag-RGO-Au platform involved detecting tumor cells in the presence of human tumor cells,

liver cancer cells (BEL-7402), and normal human liver cells (202 liver cells). This configuration facilitates the generation of robust and adjustable electromagnetic fields, fostering intense SERS signals within this confined space. The distinct Raman spectrum alterations observed when labeled tumor cells were placed onto the Ag-rGO-Au substrate suggest potential indications for identifying tumor cells. Xie and coworkers employed a label-free SERS biosensor based on graphene/gold nanopyramids to detect colon cancer p53 −/− cells among p53 +/+ colon cancer cells[83]. This hybrid SERS biosensor could discern p53 −/− cells from p53 +/+ cells across three distinct cell states, live, dead, and burst, with an average sensitivity of 81% and specificity of 97%. These findings underscore the potential utility of this graphene hybrid SERS system in cancer detection. In addition to these insights, it is important to underscore the relevance of other review articles within this domain[84-86]. These complimentary reviews offer perspectives on various aspects of graphene-based biosensing, ranging from its fundamental properties to its diverse applications in biomedical diagnostics and beyond. Collectively, these articles contribute to the comprehensive understanding and continuous advancement of graphene-enabled biosensing technologies.

Interactions of graphene nanosheets with blood-related entities such as plasma proteins, blood cells, and blood-borne parasites have been widely studied in the biomedical field. The large 2D aromatic surface of graphene combined with functionalize surface makes it a potential substrate for biomolecular interactions. Kenry et al. investigated nano-bio interactions between blood plasma proteins and three types of carbon nanomaterials (graphene and GO)[87]. By utilizing Raman spectroscopy and other techniques, they concluded that carbon nanomaterial's morphology, surface oxygen content, protein structure, and hydrophobicity play a major role in nano-bio interactions. In another study, Lu and coworkers reported that a globular protein, β-lactoglobulin (BLG), can be decorated on rGO to obtain a BLG-rGO composite that has pH sensitive solubility[80]. Gold nanoparticles were also integrated into BLG-rGO composite to develop an SERS substrate with larger enhancement factors in biological sensing. In a different study Kim et al. demonstrated that a minimum of three graphene layers is necessary to avoid underlying substrate and water effect on peptide adsorption . By analyzing uniform and nonuniform graphene with varying numbers of layers, the further verified that the quality of graphene influences biomolecule−graphene interactions and that the binding of peptides to the graphene surface in aqueous media strongly depends on the graphene−peptide interaction rather than graphene−substrate interactions.

Based on the above findings, it's obvious that graphene and its derivatives play following multifunctional roles in different biosensing platforms. (a) as a fluorescence quencher (b) as a high thermal conductive substrate to dissipate heat (c) as a 2D scaffold to fix interfacial assembled nanoparticles (d) as chemical mechanism source for SERS enhancement (e) as a representative substrate to facilitate molecule binding through π-π interactions and (f) as a nano spacer for other nanoparticles. Overall, understanding graphene-molecule interactions will provide an important insight into performance optimization of graphene-based biosensors. To date, graphene-based substrates have detected various biomolecules such as proteins, DNA, bacteria, nucleoside and biomarkers for disease progression. These have shown great promise in practical applications such as cancer diagnosis, bioimaging, photothermal therapy, and drug delivery that can promote personalized medicine and new possibilities for diagnosis and treatments. Proper surface functionalization and tuning parameters such as layer number, Fermi level, and laser wavelength graphene could improve biocompatibility and effectiveness in end application. The exploration of graphene in real-life applications is only in its infancy, and much work needs to be done with the collaboration of material scientists, chemists, physicists, and biologists.

5 Summary

Graphene boasts exceptional electronic, optoelectronic, and mechanical properties, paving the way for various device applications. We have reviewed the recent advances in Raman spectroscopy of graphene studying its fundamental phenomena and practical applications. Raman imaging has demonstrated its capability to offer spatially distributed details regarding key properties of graphene flakes, including layer

number, stacking order, edges, strain, and growth mechanism. Raman spectroscopy is a pivotal and non-destructive characterization technique, offering real-time and online capabilities for monitoring property alterations and tailored functionalities in graphene-based materials within a diverse range of devices. These encompass technologically significant domains such as Field-Effect Transistors (FETs), energy storage components, photovoltaic cells, Organic Light Emitting Diodes (OLEDs), Nano-Electro-Mechanical Systems (NEMS), and the burgeoning field of graphene-based van der Waals (vdW) heterostructures. Its capacity to provide intricate insights into the structural and chemical attributes of graphene materials makes it an indispensable tool in advancing these technologies.

This comprehensive review is an essential guide for researchers and professionals engaged in fundamental research, and the development of industrial applications involving graphene-based materials. By offering a detailed exploration of Raman spectroscopy's manifold applications and potential, this review seeks to equip scientists with the knowledge and methodologies necessary to navigate the complexities of characterizing and optimizing graphene-based materials for cutting-edge applications. Additionally, it aims to foster an enhanced understanding of the intricate interplay between material properties and device performance, thereby contributing to the continued advancement of this dynamic and rapidly evolving field.

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