Recent Progress in Using Graphene as an Ultrathin Transparent Support for Transmission Electron Microscopy

Sapna Sinha* and Jamie H. Warner*

Transmission electron microscopy (TEM) has long been used as the ultimate characterization technique for nanomaterials at the atomic level. Herein, the importance of the use of graphene in TEM is also presented to introduce the properties that make it indispensable for the characterization of other nanomaterials and study their properties and van der Waals interactions. A broad overview of the importance of using TEM for the study of nanomaterials and the rise of graphene as a superior substrate for the study of different kinds of low-dimensional materials is provided. A review of the study of morphology, properties, and behavior of a range of nanomaterials is presented, with a specific focus on how graphene facilitates these studies due to its unique influence and interaction with the specific materials under TEM. This review presents an overview of the various studies and characterization that have been carried out on a range of nanomaterials using TEM with the use of graphene, and discusses the future challenges and engineering applications.

1. Introduction
With increasing interest in nanomaterials and small molecules, there have also been rapid developments in various imaging and characterization techniques used to study their properties, such as noncontact atomic force microscopy (nc-AFM),\(^1,2\) scanning tunneling microscopy (STM),\(^3\text{–}6\) etc. STM is one of the most popular tools used for imaging surfaces at the atomic scale. However, despite being heavily used for imaging molecules as well as polymers, STM is limited by sample conductivity, cleanliness, and acquisition time. Similarly, even though nc-AFM provides higher resolution to image atoms and even bondings,\(^7,8\) its long acquisition time (i.e., 15–30 min), makes it a challenge for statistical and dynamic analysis of the molecules. Therefore, one of the major characterization tools with the capability to characterize and study the atomic-level structure and properties of nanomaterials is transmission electron microscopy (TEM). State-of-the-art TEM enables imaging and studying nanomaterials with atomic resolution, while at the same time offers various other ways of characterization such as energy-dispersive X-ray spectroscopy (EDS), electron energy loss spectroscopy (EELS), etc. TEM has witnessed rampant development in the past decades, which was timely with the development in the field of nanomaterials, in the sense that it allowed aberration correction to operate the microscope at high resolution, even at lower acceleration voltages, so that the damage to the nanomaterials is minimized.\(^9\) Both TEM and scanning transmission electron microscopy (STEM) are extensively used for the characterization of nanomaterials\(^10\) because they are reliable, while at the same time, offer various other ways of characterization, such as elemental analysis through energy dispersive X-ray spectroscopy (EDS) and EELS.

With the advancement of TEM and improved resolution and capabilities in imaging, the bottlenecks for obtaining high-resolution images have been shifted from the instrument to sample preparation. Therefore, it is increasingly being recognized that the optimization of sample preparation technique as well as minimizing the background noise from the sample support is very important for obtaining the high-resolution images and study nanoparticles and atoms. Since the discovery of graphene in 2004, it has spawned massive interest in its 2D sp\(^2\)-hybridized carbon structure.\(^11\) A single sheet of graphene is comprised of a single-atom thick carbon layer with regular and periodic structure. Its excellent electrical properties were first highlighted by Novoselov et al.\(^12\) and since then, a tremendous amount of research has been conducted to study its morphology, defects, electrical, and mechanical properties, etc. An increasing number of applications of graphene have also emerged in the past decade, exploiting its exceptional properties.\(^13\) TEM is one such area where graphene is proving its worth as a sample support for electron microscopes.

2. Graphene—Excellent Properties as a TEM Substrate
Graphene is the thinnest possible material with high crystalline morphology. A purely crystalline, pristine graphene is also electron “transparent” to TEM up to the resolution of 2.13 Å, which is the resolution obtained by many cryo-TEM.\(^14,15\) This is due to
the fact that crystalline periodicity of 2.13 Å is higher than the periodicity of graphene, thus making it an effectively “transparent” substrate. Moreover, the transmission coefficient of graphene can vary a lot, effectively making it transparent at specific accelerating voltages. The single atomic thickness of 0.34 nm also contributes to very little background noise and even when the lattice is completely resolved, the signal can be easily filtered through its fast Fourier transform (FFT), if necessary.[16,17] This makes it easy to focus and study other nanomaterials on its surface, because either the graphene is completely invisible at higher voltages, or otherwise its lattice can be completely filtered out from the images. Graphene is also known to be remarkably strong, both mechanically and elastically,[18–20] which gives it the ability to withstand the high vacuum conditions inside the TEM. Moreover, it is known to be a good electrical conductor, with a conductivity six orders of magnitude higher than that of amorphous carbon, and therefore does not charge up from the e-beam.[21–23] Comparing conventional amorphous carbon and graphene as a substrate for TEM grid, graphene is one-atom thick while the amorphous carbon sheets can range anywhere between 3 and 20 nm in thickness. Moreover, the chemical inertness of graphene makes it less reactive than the amorphous carbon substrates at lower accelerating voltages. In addition, as discussed earlier, crystalline graphene can be easily masked by Fourier filtering of the images, which is not possible for amorphous substrates. The conductive properties of graphene give it the additional advantage of possessing a homogenous surface potential that reduces phase distortions of the electron waves.[24] Monolayer graphene oxide (GO) is another related material that is widely used as a substrate for microscopy. In the beginning, when it was still difficult to synthesize and transfer large areas of monolayer graphene, GO could be prepared easily and also transferred onto different kinds of substrates. However, although it has atomic layer thickness, it has limited electrical conductivity that is dependent on the degree of oxidation.[25]

With the advent of chemical vapor deposition (CVD) synthesis of graphene,[26] large-area graphene can be synthesized and transferred easily as a substrate for TEM purposes, thereby alleviating the limitations inherent to the use of GO membranes. The availability of large area graphene allows for the easy transfer of graphene from copper substrate to TEM grids, which result in freestanding graphene,[14] providing ample surrounding space with sufficient contrast for tuning the microscope and focusing away from the region of interest. It is worthwhile to note here that multilayer graphene (MLG) is much easier to synthesize and thus more readily available. However, although MLG has superior stability, it is often more rigid and contaminated than the single graphene layers. Therefore, though MLG is still far better than amorphous carbon or GO substrates, single-layer graphene is often preferred for its clean and flexible surface (Figure 1).

Another membrane which has been popularly used for imaging individual nanoscale particles or molecules is carbon nanotubes (CNTs). The development in the area of CNTs preceded that of graphene, and thus a lot of in situ TEM research using CNT substrate was conducted much earlier on.[27] Molecules, ions, or atoms can be easily confined inside CNTs, which can be exploited to use it as a nanocontainer to study the properties of individual species in one dimension. CNTs are strong, thermally stable, and mechanically robust[28] and can be atomically thin, which facilitates the study of the molecules directly, in real space through the nanotube wall. In the past 20 years, dozens of different types of nanomaterials have been encapsulated across the mesh of CNTs and subsequently studied using TEM.[29] such as molecules,[30,31] nanoparticles,[32] fullerenes,[33] organic or biomolecules,[24] etc. Extending the application of CNTs beyond nancontainers, it has also been used as a chamber to carry out nanoreactions.[35] CNTs provide highly efficient platform for complex molecules and its highly stable and robust nature enables control over the encapsulated nanomaterials while providing the real space for imaging and studying the chemical reactions at a molecular level.[16,37] The mechanical robustness, high thermal stability and chemical inertness of CNTs allow reactions to be carried out inside the tube under harsh conditions without the CNTs getting damaged or participating in the reactions themselves. Khlobystov group in Nottingham have long explored this phenomenon, called chemTEM, which follows the chemical transformations at the single-molecular level with the electron beam inside a TEM.[38] However, the limited space and only one accessible direction of motion in CNTs does not provide any information on the behavior of molecules in two dimensions. In addition, the limited space, harsh insertion process and the curved shape of CNTs reduces their applicability. Graphene overcomes these factors as it provides a 2D surface for the free motion of nanomaterials on the surface, as compared with CNTs that provide only one degree of freedom of motion. Moreover, nanoparticles can be easily transferred on top of graphene, as simply as just drop-casting them from solution at room temperature.

One of the limiting factors of using graphene is the radiation damage, which is a detrimental side effect of using electron beam in TEM. The damaging factors may include ionization, heating, chemical etching, and/or knock on displacement. However, a series of experiments conducted by Meyer et al. showed that these damages can be controlled or substantially reduced by controlling the beam current applied to the sample and the energy of the electrons used. Beam damage in pristine graphene has been reported to be completely suppressed at electron acceleration below 80 keV.[39,40] Under 80 keV electron irradiation, defect-free graphene can sustain its perfect lattice structure up to very high doses.[41,42] Moreover, although high electron acceleration in TEM is known to create damage in the material, it has been reported that graphene can effectively self-repair ion damages in the temperature range that are currently been used for its CVD synthesis.[43] Therefore, when in situ heating of graphene at this temperature range is carried out in the microscope, it can effectively self-repair the damages done by the electron beam. This shows its durability and its importance as the ultimate thin sputtering shield under electron beam irradiation. Aside from conventional TEM, the graphene supports are also starting to get used in in situ TEM to enable the imaging and investigation of other specimens. Transitioning from 3D bulk structures to low 0D or 2D materials has a significant influence on the nature of damage under the electron beam.[44] Therefore, graphene encapsulation of nanomaterials is yet another area which has grown tremendously to study beam-sensitive materials in TEM.

The graphene membrane thus provides the ultimate sample support for TEM, and has helped refresh experimental TEM approaches[45] and renewed interest in TEM supports[46] The application of graphene as a sample support in TEM has been
demonstrated widely in the literature, especially with the advent of aberration correction in TEM. McBride et al. and Meyer et al. reported the first application of few-layer graphene films for nanocrystal imaging. Their studies showed the high sensitivity that graphene membranes provide in TEM to facilitate imaging of sub 2 nm CdSe nanocrystal and that it can also be used to detect adsorbates as light as hydrogen atoms using TEM with nearly background-free imaging. It was shown that lower carbon background from graphene particularly benefited the study of the CdSe using EELS. The results show that graphene supports could help investigate the dynamics of small nanoparticles and molecules in real time and provide insights into complex chemical reactions. Although the results were based on few-layer graphene, it opened doors to many possibilities. Now that it is feasible to prepare single-layer large-area graphene, more research has been conducted in this direction, and graphene specimen support technique for TEM has been established. Thus far, graphene sheets have been used as a support for TEM studies of nanoparticles, organic molecules, biomolecules, etc., which has been discussed in details in the next sections.

The use of graphene as a substrate in TEM can be widely divided into five categories—1) Imaging the adsorbates and light-weight atoms by themselves and their interaction with graphene. 2) Use of graphene as an inert and mechanically stable substrate in in situ experiments. 3) Straightforward use as a substrate for high-resolution imaging of nanomaterials, which cannot be otherwise obtained in a freestanding form for TEM observations. 4) As an encapsulation cell for imaging highly electron beam-sensitive materials like biomolecules, organic compounds, etc. 5) As a unique material to lead research toward creating and studying hybrid interfaces with other materials or specimens with the use of electron beams, by exploiting the presence of dangling bonds from carbon residues on the surface.

The objective of this Review is to systematically present the various facets of nanomaterial research which has been facilitated using graphene as a substrate in electron microscopy. We discuss the various low-dimensional materials and understand their van der Waals interaction with graphene under TEM and present an outlook to carry out future research.
3. Microscopy with a Graphene Substrate

In this section, we explore the different ways that graphene has been beneficial for microscopy. The section has been divided into two parts for simplicity. The first section is a comprehensive review of different kinds of materials that have been studied using graphene and the second section reviews the different functionalities and applicability of graphene for studying materials under microscope.

3.1. Study of Nanostructured Materials

3.1.1. Fullerene study and encapsulation

The study of 0D materials, such as fullerenes, is a significant area of nanomaterials research that has benefitted from the use of graphene in TEM as a substrate. Carbon supports that are only a single-atom thick provide ideal surfaces to study and serve as encapsulation containers for studying fullerenes. Since the discovery of CNTs in late 20th century, single-walled carbon nanotubes (SWNTs) have been used as a molecular test-tubes or nanoreactor capsule to encapsulate fullerenes to study it, within high-resolution TEM, with reduced radiation damage. Numerous studies have been conducted on empty cage fullerenes and endohedral metallofullerenes (EMFs) inside SWNTs, studying their synthesis, structure, dynamics, reactions, defects, and damage. However, the CNT is a 1D system that only allows studying the dynamics or reactions of fullerenes in a singular dimension. Graphene on the other hand, provides a similar environment with a single atom thick encapsulation for fullerenes, while at the same time, offering them two degrees of freedom. Graphene encapsulation on small metal particles colloidal nanocrystals, or other 2D materials systems have shown to reduce the rate of radiation damage. Extending that encapsulation process to fullerenes gives fullerenes a similar protection from radiation while at the same time, providing a reaction chamber that is less constrained than a 0D fullerene cage or SWNT test tubes. This provides new avenues for the study of fullerenes, their reaction and dynamics on a large flat surface.

Choe et al. used aberration-corrected TEM to demonstrate that the C60 molecule spin coated on graphene can migrate and form stable anchoring onto graphene point defect sites. Molecular rotational motion of anchored C60 on graphene can be monitored at a single molecular level. The interaction of C60 with graphene also leads to preferential registry of the rotation (Figure 2a-c), which they explained through the stacking-dependent energy landscape between the two systems. Moreover, it was also shown that the energy barrier for rotational-angle configurations could be estimated by simply analyzing the molecular dynamics of the fullerenes. They carried out another investigation on the structural disordering and heterogeneous dynamics of C70 deposited on graphene (Figure 2h–l). Their experiments showed interesting results on e-beam induced first-order transition-like crystal melting of the disordered C70 molecular system on graphene, as shown in Figure 2m. The observed heterogeneous dynamics of C70 after e-beam radiation bore similarity to the dynamical heterogeneity in supercooled liquids that are close to their glass transition. The unprecedented real-time high-resolution imaging, opens doors to completely new possibilities of using various types of fullerenes and EMFs on graphene to study molecular glass and supercooled liquids. However, the images obtained of the C60 on the graphene are not as clear and the e-beam defect induced on C70 was easier to obtain because of the lack of encapsulation. This encapsulation process and its effect was recently studied by Mirzayev et al. by fabricating 0D/2D heterostructure that incorporated C60 molecules between two graphene layers to form a buckyball sandwich (Figure 2d,e). They showed interesting results on clean and ordered C60 islands with one molecule thickness incorporated between two graphene sheets. The graphene layers allowed the study of the structure and dynamics of C60 at the atomic resolution, and at the same time proved to be successful in shielding C60 molecules from radiation damage during STEM imaging (Figure 2d–g).

Prior work conducted in this area showed that Gd-based metallofullerene (Gd3N@C60) molecules could be used to create single adatoms and nanoclusters on a graphene surface. An in situ heating holder within an aberration corrected scanning TEM was used to track the adhesion of EMFs to the surface of graphene, Gd metal ejection, and diffusion across the surface, as shown in Figure 2n–r. The opening of MFs causes cargo release of dopant adatoms on the graphene surface without chemically etching of the underlying graphene or damaging the sample itself, providing an excellent way to create doped graphene monolayers. The results showed that the metal ejection dynamics was a result of in situ heating rather than e-beam-driven mechanism. TEM proved to be a useful technique in detecting and capturing the dynamics and initial disintegration process as soon as the temperature was increased to 500°C. In this case, our method can potentially be used for drug delivery and imagine where graphene can act as a host to introduce the Gd atoms. Whether anchoring the fullerenes to the graphene surfaces via defects/chemical addition, or depositing them on top, or encapsulating them on either side with graphene, all possibilities show interesting results and help investigate their crystal phase and motions at low and high temperatures. Further research in the area can provide valuable information on the dynamics and energetics for various types of fullerenes, which have not been studied before and potentially open doors for novel applications.

3.1.2. 1D Materials

CNTs have long been used as a substrate for encapsulating and studying individual molecules, atoms, and even 1D networks such as organic frameworks, atomic chains, polymers, etc. However, the size and diameter of CNTs limit the types of 1D networks studied inside this container. To overcome these limitations, graphene has now slowly started to be used as a substrate to visualize and study 1D networks such as nanowires or 1D frameworks on a 2D surface, without needing to encapsulate them in a limited space provided by the CNTs. Metal nanowires (MNWs) are a good example where some work has been carried out in both integrating the material with graphene and also on exploiting graphene as a substrate for their studies. Such nanowires can be easily transferred on top of graphene...
Figure 2. (a–c) Sequential TEM images of rotating C60 molecules anchored on graphene. The white and yellow dashed lines represent the graphene zigzag and armchair directions respectively. Scale bar corresponds to 1 nm. (d–g) Monolayer area of a C60 sandwich between two graphene layers showing both monolayer and multilayer regions (d), higher magnification of monolayer region (e), FFT of (e) with graphene shown with red circles, and some fullerene shown in yellow circle (f) and schematics of a buckyball sandwich with top and side view in (g). (h–i) Atomic models of C70 deposited on graphene before and after structural transition under e-beam, respectively. (j–l) TEM images of C70 arrangement showing increasing structural disorder from (j) to (l) on prolonged exposed to e-beam. Scale bar corresponds to 2 nm. (m) Sequential TEM images taken in the first 60 s of e-beam exposure, showing the structural transition from molecular C70 crystal phase (enclosed in blue) to liquid phase (enclosed in red). The disordered molecular regions of C70 come together slowly while showing apparent phase boundaries with the initial homogeneous crystalline C70 phase. Scale bar, 2 nm. (n–r) Sequential ADF-STEM images showing an individual Gd3N@C80 attached to the edge of the carbon layer on graphene, which fuses and releases Gd atoms. The time frame between the images is ≈20 s, while heating in situ at 500 °C. (a–c) Reproduced with permission.63 Copyright 2016, Royal Society of Chemistry. (d–g) Reproduced with permission.65 Copyright 2017, AAAS. (h–m) Reproduced with permission.64 Copyright 2019, Springer Nature. (n–r) Reproduced with permission.66 Copyright 2018, American Chemical Society.
through spin-coating method and subsequent heating can help them weld together to form a metal networks and mesh on top of graphene.[68] As shown in Figure 3c.d, clean and thin graphene substrate also makes it possible to carry on elemental analysis of the elements of the welded nanowires. CNTs themselves have been studied on top of graphene substrate. CNTs have been shown to be both anchor on top of graphene vertically or integrated into graphene horizontally to form hybrid material.[69] Anchoring of CNTs on graphene is of particular interest as the basic physical phenomenon allowing anchoring is not van der Waals forces but active anchoring on the site via a hole on graphene, presence of an atom or careful chemical synthesis of such integrated hybrid system.[69,70] Such integration of graphene with CNTs, Figure 3f–g have also shown increased graphene film toughness.[69] With that said, although graphene can act as an excellent substrate, it is important to note that most of the recent research in these areas have been carried out with reduced graphene oxide (RGO) instead of pure crystalline graphene.[71] The reason for which is the tunable electronic properties of RGO which make it an excellent candidate to form composite hybrid with nanowires to enhance their electronic properties. RGO then also acts as a substrate for such hybrid materials, which removes the necessity of using graphene. 1D nanowires is one field where graphene has not had much contributions yet, as a substrate or as a hybrid material, and the thus, the field remains open for future investigations.

Figure 3. a–e) TEM images of AgNW on graphene film where (a) shows the low magnification and (b) welded junction of AgNWs with their (e–f) EDS elemental mappings of Ag and C, respectively. e) Edge of the graphene film in (b). f–g) Schematic illustration and high-resolution TEM images of CNT–graphene integration, respectively. a–e) Reproduced under the terms of a Creative Commons Attribution 4.0 International License.[68] Copyright 2020, The Authors, published by Taylor & Francis Group. f–g) Reproduced with permission.[69] Copyright 2017, American Chemical Society.
Other works on formation of 1D nanoparticle arrays and metal nanowires have been discussed separately in the subsequent sections where it is more relevant to discuss about their growth and catalytic properties of metal–graphene interactions.

3.1.3. 2D Materials

The advances in TEM have enabled high-resolution imaging of 2D materials, which has increased our understanding of these materials at the atomic level.\(^{[72]}\) However, the electron beam, especially at accelerating voltages above 80 kV, causes irradiation damage such as breaking of bonds, mass loss etc., yielding difficulties in studying the intrinsic properties of the 2D materials. Moreover, lower voltages lead to reduced resolution and more difficulty with aberration corrections in recording the information required. Incorporating graphene with these 2D materials provides them with an ideal substrate as well as encapsulation during TEM imaging, thus facilitating irradiation damage-free study of these materials.

Dozens of different techniques have been reported in the past decade for fabricating 2D lateral heterostructures to facilitate study of their intrinsic electrical properties.\(^{[73]}\) However, little study has been conducted to encapsulate 2D materials with graphene to study their structural morphology via TEM. In some cases, it has been shown that encapsulation eliminates damage, almost entirely.\(^{[60]}\) facilitating clean atomically resolved morphology of the intrinsic structure. In addition, “sandwich” structures can also enhance recombination rates, because of reduced atomic diffusion, which is also said to be the rate-limiting step of the damage\(^{[74]}\) within the atomic structure of the 2D crystal. Zan et al.\(^{[61]}\) showed that detailed understanding of the crystallography, reconstruction, and stacking order of 2D atomic crystals and their heterostructures can only be studied through direct imaging and identification of each atom in a protected environment. They showed that MoS\(_2\) crystals have the highest durability and lowest defect formation within two graphene layers (i.e., three-layer stack of graphene/MoS\(_2\)/graphene), as compared with when they are left pristine or placed on top of a graphene substrate, and it also allows use of TEM to carefully control the nature of formation of defects in the crystal. The results demonstrate that both knock-on damage and ionization effects induced by the e-beam can be reduced dramatically, allowing defect-free chemical analysis of the material. Quantitative experiments were conducted by Algarra-Siller and coworkers on using different configurations (Figure 4a) of graphene protective layers on MoSe\(_2\)\(^{[75]}\) where they found that protective layers could provide up to 600-fold reduction in the rate of damage with total encapsulation (Figure 4a) bringing ideal protection. Interestingly, they also found that although graphene protective layer on top of the 2D crystal (Figure 4a) would be expected to reduce the inelastic damage to the material, but having it underneath the 2D material (Figure 4c) led to more elimination of damage, to up to 24% more. They attributed the phenomenon to reduction of the surface sputtering which is generally caused by the direct knock-on damage from the e-beam. Similar results of WS\(_2\) on top of graphene has been reported by Kim et al.\(^{[76]}\) where they showed that simply utilizing the graphene as a substrate, instead of encapsulating it completely, can also result in substantial improvement in long-term stability of the material.

Graphene has also been used as a substrate to study laser irradiation to directly anneal or ablade palladium diselenide (PdSe\(_2\)) thin films using high power, or trigger the local transformation of PdSe\(_2\) into a metallic phase PdSe\(_{2-x}\). The laser-dependent study was conducted on the PdSe\(_2\) film suspended both with and without graphene and the material was studied using ADF-STEM afterward, Figure 3b. High laser power resulted in local material degradation and formation of Pd nanoparticles (NPs), whilst lower laser power was demonstrated to be able to controllably modify the PdSe\(_2\) phase into Se-deficient PdSe\(_{2-x}\) phase. The presence of graphene underneath has interesting effect on the amount of transformed sample. It was observed that by varying the laser power, the degree of material damage changed substantially between the PdSe\(_2\) samples which were supported by graphene and which were not. For instance, the laser damage, i.e., the hole, could be easily induced at 60 µW laser power, into PdSe\(_2\) film suspended across vacuum, whereas no damage was seen for PdSe\(_2\) suspended on graphene film, as shown in Figure 4b. These results were consistent with thermal energy transfer to underlying graphene layer which results in lower local temperatures within PdSe\(_2\) film, as discussed earlier. Furthermore, the presence of graphene helped to support the detached Pd nanoparticles that would have otherwise disappeared from the sample, Figure 3c.d. This helps to fully understand the laser-induced transformations.

It has also been shown that different kinds of analysis particular to the 2D crystals can be conducted within TEM without damage to the crystal, such as crystal orientation,\(^{[78]}\) EELS,\(^{[61,79]}\) EDS,\(^{[80]}\) and elemental mapping,\(^{[80]}\) with easy elimination of signals from the protective graphene sheet. Graphene has also been shown to act as a protection layer to assist layer-by-layer thinning of phosphorene samples using TEM, rendering high-quality monolayer and bilayer regions.\(^{[81]}\) They also showed that phosphorene shows increased stability and reduction in radiolysis damage when exposed to e-beam. Graphene has also been shown to have influenced the epitaxial alignment of 2D materials, which is attributed to the van der Waals interaction between the two materials. For instance, when amorphous MoS\(_2\) was deposited on graphene, it showed restructuring into crystalline domains when irradiated with electron beam.\(^{[82]}\) Moreover, graphene can also have a strong impact through the van der Waals interaction with 2D materials, which is particularly important for future applications in optoelectronics. Our group utilized high-angle annular dark-field STEM (HAADF-STEM) to show that monolayer lead iodide shows a preferential alignment and structural morphology on graphene, as shown in Figure 4e-f.\(^{[83]}\) PbI\(_2\) was observed to be energetically most stable in 1H structural phase when on top of graphene, instead of the otherwise reported 1T structural phase. In addition, we also showed that strong epitaxial alignment of PbI\(_2\) monolayers with the underlying graphene lattice occurs, PbI\(_2\) zigzag matching graphene arm-chair direction, leading to a phase shift from the 1T to 1H structure to increase the level of commensuration in the two lattice spacings (PbI\(_2\) and graphene). Such results provide insights into the influence of graphene as a substrate on the structural morphology of other 2D materials.
3.1.4. Biological Materials

Thus far, carrying out TEM of biological cells has been an important challenge in research. Biological cells are known to be hygroscopic, permeable, as well as electron-absorbing, thus leading to a structural decay when exposed to the electron beams.[84,85] Also, when imaged under high vacuum condition, the reduced pressure leads to contraction in size and volume of the wet cells as well as electrostatic charging in TEM.[86] With the recent use of graphene in TEM, Mohanty et al. showed that the problems mentioned earlier can be solved by encasing the beam-sensitive biological cells in graphene. Graphene is not only electron transparent, but its high electrical[87,88] and thermal conductivity[88,89] facilitates imaging and protection of fluidic samples without electrical charging or heating of the cells. Mohanty et al.[90] showed that bacteria cells encased within graphene layers could be imaged well under TEM, as shown in Figure 5f, while withstanding very low pressure, i.e., $10^{-5}$ Torr, as well as high beam current up to 150 A cm$^{-2}$. Pantelic et al.[14] showed that positively stained DNA can be imaged in high contrast under STEM using pristine graphene which is difficult to achieve with the use of conventional substrates such as amorphous carbon or GO.

Methods have been reported in literature which can assist biological molecules mount on the hydrophobic surface of graphene. Cerf et al.[91] introduced a solvent mediation method to introduce biological molecules from PDMS stamps to the hydrophobic graphene. With this method, they showed that even single plasmid DNA, which is made up of only light atoms (C, N, O, and some H and P), can be imaged as well as arranged across graphene, and STEM can be used for a complete genetic and epigenetic mapping by reading single molecule system. In a different form, Pantelic et al. showed that functionalization of graphene by oxygen doping, can render it hydrophilic, and can help mount the biological samples well, while at the same time keeping the other properties of graphene intact, thus facilitates imaging under TEM.[92] Similarly, other types of modification of graphene have also been reported to assist the mounting of biological samples on the material, such as hydrogenation,[93] nanopore drilling[94–96] noncovalent aromatic functionalization,[97] bioactive-ligand functionalization.[98]

Progress in this area would especially benefit the study of biological samples on molecular scale via cryogenic electron microscopy (cryo-EM), where the embedment of biological molecules on the substrate surface is necessary. Furthermore, additional work in this area can lead to fine tuning of graphene properties to allow better control over the quantity and orientation of the biological molecules on the graphene surface for microscopic studies. An important thing to consider in this...
The process is that residues can result on the surface of graphene after the functionalization process. Therefore, care must be taken to clean the surface properly after the functionalization and take into account any residual material while imaging or data analysis.

The use of graphene as a transparent conductive support in TEM for studying nanobio materials has been extended and shown to work exceptionally for many different specimens, such as viruses, insulin amyloid fibrils, unstained DNA, DNA-assembled Au nanoparticles and protein complexes, as shown in Figure 5. The extraordinarily large area that graphene provides gives ample space for carrying out purposeful reaction with living organisms. Graphene can interact with the biological molecules, opening up a whole new field of science impacting the biological systems. With the use of STEM, Kabiri et al. imaged both stained and unstained DNA origami nanoplatelets (Figure 5g) on graphene as well as amorphous carbon. They addressed that electron-induced contamination was not observed on the graphene substrate as compared with the amorphous carbon substrate and it also showed less sample drift, charging, and high contrast. However, DNA origami nanoplatelets were observed to show structural disorientation on top of graphene. This can be attributed to the π-π interaction of the DNA with the substrate underneath, which points toward interesting field of science where the biological molecules can interact and form new structures with graphene. This also opens-up interesting area of studying interactions of biological samples by functionalization of the graphene substrate. Progress in this direction would also lead to real-time imaging of biological samples as well as potentially also help study their biochemical activities under controlled conditions.

Figure 5. a–e) Dark-field STEM images of fibrils taken at 30 keV on top of a graphene film in (a) and a 20-nm carbon film in (b). Insets in (a,b) show the line profiles along the white lines in the image. The use of graphene substrate increased the signal-to-noise ratio and increasing the visibility of thinner films. c,d) STEM image, SE image, and schematic respectively of fibril on graphene showing the twisted-ribbon structure. Scale bars are common between (a) and (b), and between (c) and (d). f) TEM image of wrapped bacteria (WB) on graphene. Inset shows the lateral cross section of the WB. About 90% of the bacteria was found to be completely wrapped. g) STEM image of DNA origami on graphene. d) Schematic of origami on graphene (not to scale). The arrows show the different orientations of graphene origami and where the electron beam first hits the surface. No difference was observed in terms of damage response. a–e) Reproduced with permission. Copyright 2017, Elsevier Ltd. f) Reproduced with permission. Copyright 2011, American Chemical Society. g) Reproduced with permission. Copyright 2017, Wiley-VCH.
3.1.5. Organic Compounds

A longstanding challenge in characterization of organic compounds is to understand their distribution and be able to image atomic structures of single molecular compounds which are usually made up of light elements. Graphene serves as an excellent substrate by providing transparent and large surface ultraclean area to image them and to study their mobility and structures of their conformers. Janicek et al.\[36\] showed that EELS can be used in STEM mode of imaging to directly visualize and quantify ligand distributions on gold nanorods, when deposited on graphene. The results showed ligand binding densities between individual nanoparticles and the impact of electron spectroscopy to understand the molecular distributions. Ke et al.\[107\] showed that a single Ru4POM (polyoxometalates) molecule on graphene depicted dynamic rotation without any influence from the graphene surface, and can be monitored by time sequence TEM studies. The authors showed that both the morphology and the reactivity of Ru4POM-graphene hybrid can be tuned by the right selection of functionalization. The study is key to understanding the nanographene modification and applications. Similarly, other research has been conducted on different kinds of organic compounds and ligands on graphene, such as mobility of single polyoxometalate ions,\[108\] monomolecular transformations of perchlorororonene (PCC) molecules,\[18\] characterization of magic-sized CdSe and CdTe nanocrystal ligands,\[109\] molecular reactions on graphene,\[146\] etc. Another way of studying these large organic molecules is by anchoring them to the surface of graphene. Merkevich et al.\[110\] reported that the structure and dynamics of PCC can be studied using e-beam to anchor it on graphene. When the molecule is dropped on graphene it initially forms a face-on orientation on the surface, but after being irradiated for a few to hundreds of seconds (doses between $2 \times 10^6$ and $13 \times 10^3$ e$^{-}\text{nm}^2\text{s}^{-1}$), the molecules stand up vertically on the surface. The e-beam initiates a two-step chemical reaction where one of the C–Cl bonds in PCC break to form a covalent bond with C from graphene surface. Further in situ experiments on this system was conducted by Chamberlain et al.\[18\] and Skowron et al.\[146\] to deduce that Diels–Adler reaction takes place with PCC and graphene on the surface when irradiated with e-beam. Further experimental work on e-beam-driven organic-graphene reactivity could help develop hybrid materials with new functionalities.

Recently, the use of individual heavy metal atoms as a marker in studying complex structural change of organic molecules has been demonstrated. HAADF-STEM is an excellent imaging mode for identifying and monitoring the heavy metals in the organic molecules as they appear as bright spots during imaging, and graphene can provide transparent support to easily identify and study the transient molecular movement of these heavy metal atom-decorated single molecules. Gerkman et al.\[111\] studied photoisomeric azobenzene derivatives on graphene support and were able to identify photoisomeric states of the molecule, as shown in details in Figure 6a–k. Kyung Lee et al.\[112\] also showed the similar use of heavy metal Pt-atom markers in linear porphyrin hexamer (Pt-L6) for studying the 2D monolayer self-assembly of the molecule on graphene. The results demonstrate how the metal markers in complex organic molecules on top of graphene can be exploited to study large-scale structure, alignment, chain bending, and packing at individual molecule level (Figure 6i–r). Incorporating such HAADF-STEM imaging has potential to study dynamically changing device structures incorporating both organic molecules and 2D graphene.

Not only big organic compounds and ligands, but also single lightweight molecules have also been studied on graphene using TEM. In fact, TEM of the dynamics of molecular-scale adsorbates on graphene has been studied well since the beginning of research in graphene, which is not unusual, since studying molecules did not require as much of high resolution as does the study of single atoms.\[113\] Much research has been carried out into characterizing the behavior, stability, and dynamics of different kind of organic molecules on graphene surface, such as carbon and other light adatoms.\[16\] One of the earliest studies described the structural reorganization of hydrocarbon adsorbates on graphene to initiate crystallization, to form amorphous carbon monolayers, through in situ heating above 1000 K. In this investigation, Westenfelder et al.\[114\] showed that graphene not only served as a quasitransparent substrate, but also as an in situ heater. The physisorbed hydrocarbons later formed polycrystalline graphite at temperatures above 2000 K. Other studies have also been carried out on small molecules such as H, O, NH$_4$, etc. to predict their mobility, diffusion, and dynamics on graphene.\[115,116\] Such studies have the potential to lead the way to understanding diffusion barriers and chemical activity of single molecules on graphene for applications in graphene-based catalysts.

3.1.6. Molecular Self-Assembly

STM studies have shown that the deposition of organic molecules on graphene can either lead to formation of well-ordered organic layers which are influenced by the epitaxy of graphene, or lead to charge transfer between the two materials.\[117\] The use of molecules and exploiting their self-assembly is a fast and scalable way to realize nanoscale architectures with tailored properties. A lot of research has been conducted on using graphene as a substrate to predict and control the synthesis of these 2D supramolecular organization.\[118\] Graphene can provide the 2D domain to control the surface-confined molecular states and their self-assembly. However, most of the research, which have been conducted in the field in the past several years, have utilized AFM or STM as the characteristic tool. Compared with other characterization techniques, imaging via high resolution TEM (HRTEM) can be used to study nanometer-sized materials as well as very thin samples. With the use of clean and transparent graphene substrate, the organic crystals and thin films can stay suspended during imaging. Here, the review will present the atomic-level studies conducted on these self-assembly of molecules on graphene via high-resolution TEM.

As we have already seen in the previous section, metals self-assemble on graphene when driven by high energy electron beam. Similarly, it has been reported that with the activation energy transferred, amorphous materials,\[119\] adatoms or clusters\[120\] can also assemble into crystalline monolayers on graphene. Moreover, Börrnert et al.\[119\] showed that with different high-resolution imaging modes, amorphous carbon can show different behavior on graphene under 80 keV electron radiation.
They showed that in the HRTEM mode, the amorphous carbon on graphene graphitizes in a planar manner due to the van der Waals interactions with the graphene underneath. However, in the STEM mode, the amorphous carbon supported on graphene evaporates off the surface or sometimes re-condenses in amorphous form, instead of graphitizing. This provides a unique opportunity to use different imaging modes for fabricating unique quasi-2D materials and explore their morphologies.

The interesting thing to note is that graphene can influence the alignment and packing structure of the organic molecules. Thin-film behavior of strained pentacene film was studied on graphene as a molecular assembly template by Kim et al.\textsuperscript{[121]} The crystals of pentacene showed directional morphology where they were mostly aligned in one direction. They also showed two distinct molecular packing structures of pentacene, including a new polymorph phase which has never been studied before. Kim et al. deduced that the unusual polymorph could potentially exhibit enhanced transport in the vertical direction of pentacene–graphene system due to the shorter vertical distance in the strained crystal. This demonstrates a possibility to study and control the packing and strain of molecular crystals on graphene. Similar results on supramolecular assembly of TPA (benzene-1,4-dicarboxylic acid or terephthalic acid) and TMA (benzene-1,3,5-tricarboxylic acid or trimesic acid) on graphene have been reported in literature.\textsuperscript{[122]} Both these molecules were found to self-assemble on graphene to form well-ordered crystals all the way up from atomically monolayer sheets to thin 3D films with thickness of several nanometers. The authors speculated the
epitaxial structure and orientation of these molecules is a result of both intermolecular hydrogen bonding as well as their interaction with graphene. In a subsequent study carried out by Robertson et al., on metal–ligand interaction, they synthesized PbTe nanocrystals terminated with oleic acid and trietylphosphine and studied their behavior via TEM. They found that the PbTe nanocrystals self-assembled to form packed arrays of hexagonal close packing (HCP) structure. The authors then conducted ligand exchange on the surface which resulted in disintegration of the HCP crystalline structure into irregular agglomeration of nanocrystals. The results show that using graphene can help broaden our understanding of self-assembly while also facilitating controlled manipulation of their structures, tailored to our use.

3.1.7. Metal–Graphene Interaction

High-resolution microscopy, especially HAADF-STEM can be used to study activity of heavy metals, their dynamics in 2D and interactions with graphene. Westenfelder et al. found the first phenomenon of atomically resolved structural reorganization of novel gold structures on graphene upon in situ heating. They found periodic arrangements of monolayers and bilayers of gold atoms on graphene as they form rectangular atomic structure at temperatures up to 1300 K. Here, graphene served as a transparent substrate to monitor the formation of gold monolayers, in situ heater, and also as a source of carbon which led to the formation of crystalline zinc-blend structures of gold and carbon compound. Many studies have been conducted on studying the dynamics of single metals on 2D surface and their self-assembly using graphene as a substrate and in situ heater, such as, translation motion of Fe atoms on graphene, migration of individual Au ad Pt atoms, nanoclusters formation and motion of individual Pb and Te atoms, formation of Au nanostructures, hexagonal close packing of Co on graphene, and defects and interface of Si nanocrystals. Some of the structures and dynamics are shown in Figure 7. The common observation that can be drawn from all these results is that the metal particles diffuse easily on the surface of graphene to only end up being stable at either the edge of graphene or on the amorphous carbon layers on top of graphene. They also tend to aggregate to form monolayer 2D structures, either driven by electron beam or by heating.

Surface defects can be quantified by electron paramagnetic resonance (EPR) but only TEM imaging can exactly determine the type, number, and exact location of defects in the lattice structures of the metal particles. Aside from the nanocrystal formation and motion of the particles on the graphene surface, there are other phenomena that can occur between the nanoparticles and graphene, such as e-beam-driven effusion, rotation, or structural change.

Other interesting observations of metal atoms, such as crystalline-phase transition and rotations, local strain accumulation, and epitaxial orientation of self-assembled metal particles on a 2D surface (provided by graphene) have been carried out systematically using aberration corrected high-resolution TEM. A series of results on Au nanobelts on graphene were published by Xin et al. to investigate their structure, growth mechanism, and kinetics of nanoplate formation using TEM. These results provide insight for using TEM for studying behavior of metallic atoms on a plane and fabricate dimension-controllable metal nanocrystals to inspire development in the field of graphene-based nanocomposites and catalysts.

3.1.8. Nanoparticles and Clusters

Aside from single atom metals and organic molecules, a vast range of nanoparticles, clusters, and oxides have also been studied on graphene via TEM and have also been reported to show various range of phenomenon such as rotation and translation motion, alignment, effusion, etc. when irradiated by e-beam. Warner et al. showed that electron beam irradiation provides the energy to CoCl$_2$ nanocrystals to exhibit both rotational and translational motion on graphene. The authors claimed that the coalescence of the nanocrystals, when driven by the e-beam, indicated the energy imparted through the e-beam leading to heating up of the region that in turn promoted continual rearrangement and formation of uniform crystal structure. Another research conducted by the group showed Gd migration on the surface of graphene to form a 2D structure in the hole of the amorphous carbon layer on top of graphene. Similar observation of cluster rotation driven by e-beam was reported by Chen et al. on crystalline anisotropic nanoclusters. The clusters containing 6–10 Si atoms showed out-of-plane rotation and eventually lined up in rows to achieve stable Si–C bonds, as shown in Figure 8a–w. A recent study showed the formation of 2D CsI clusters on graphene using e-beam. Vats et al reported that the size and cluster formation of the 2D structure of CsI in the presence of e-beam is dependent on the number of layers of graphene used as a substrate. Atomic clusters of a few atoms, of specific molecular shape where observed to form on single-layer graphene, whereas ordered 2D CsI crystals were observed to form under the presence of e-beam on the bilayer graphene. The authors hypothesize the presence of electronic charge interaction between cesium ions and the π-electrons present in the additional graphene layer that resulted in the observed long-range ordered 2D crystals of CsI in the bilayer regions. Such graphene layer-dependent morphology and structural variation of clusters in the presence of e-beam can provide crucial insights into the electronic interaction and behavior of nanoparticles with graphene.

Aside from rotation, dynamic diffusion have also been reported in ZnO and CuO nanoparticles when they are trapped in few layer graphene folds. It is interesting to note that the diffusion process of these two nanoparticles was found to be distinctly different, where CuO transverses in amorphous phase before diffusing out of the rupture in graphene and recrystallizing outside, whereas ZnO maintains its crystalline structure throughout the diffusion process out of the graphene by gliding through the vacancies and dislocations. In addition, the crystalline phase of the ZnO changes from the wurztite to graphene-like ZnO phase after the diffusion. These results shed light upon the interface between metal oxide and graphene and their interactions with the e-beam. Apart from cluster motion and diffusion under e-beam, thin layers of other materials can also grow on graphene when irradiated under e-beam. Hong et al. showed that
Figure 7. a–l) Catalytic growth of graphene by a single Cr atom, found at the edge of graphene, as shown in a–c) AC-TEM images, d–f) image simulation of growth process represented in (g–i) and schematic atomic model (j–l). m) Sequential images showing Pt atoms diffusion on graphene at 600 °C. The region marked with ‘L’ shows 2-dimensional migration of Pt whereas the region marked with ‘E’ shows the dispersion of Pt atoms along the edges of graphene. The time frame between two images is indicated in the image. n) AC-HRTEM image at 80 keV of 1–2 nm-sized cubic structures of Au—X monolayer structures formed at 1300 K and a thicker new 3D structure growing on the Au particles, serving as a feedstock for Au. Scale bars correspond to 2 nm. o) TEM image of templated Pt multilayer continuous film about 400 nm² region of Pt showing its grain structure and grain boundaries. (p–z’) AC-TEM images showing the mobility of Fe atoms and their corresponding atomic models. Red circles or arrows correspond to the position in the previous frame. The color blue in the atomic models correspond to Fe. a–l) Reproduced with permission,[195] Copyright 2018, Springer Nature. m) Reproduced with permission.[128] Copyright 2008, Wiley-VCH. n) Reproduced with permission,[125] Copyright 2015, Springer Nature. o) Reproduced with permission,[196] Copyright 2011, Wiley-VCH. p–z’) Reproduced with permission,[127] Copyright 2013, American Chemical Society.
**Figure 8.** a–o) Sequential time-frame and schematic demonstration of ADF-STEM images recorded at 900 °C of Gadolinium atoms and clusters moving on graphene. Time between frames a–j is 20 s. k) shows a schematic model of gadolinium migration to a hole on the amorphous carbon layer on graphene, l) is the schematic model of hole and m) is the schematic model of hold after Gd filling respectively. n) shows the ADF-STEM images of the empty region indicated by the circle in (a), and o) shows the same region as (j), after being filled by the Gd atoms. p–s) Sequential time-frame of HRTEM images, taken every 20 s, of CoCl2 nanocrystals, showing their rotating on graphene. Graphene lattice has been removed by 2D FFT filtering. t–w) Si nanocluster and out-of-plane rotation of Si atoms showing small Si nanocrystals in (t) and its atomic structural model in (v), two Si atoms rotating highlighted in yellow in (u) and its atomic structural model in (w). Time duration between (t) and (u) is 8 s. Scale bar corresponds to 0.5 nm. x) Lateral growth of ZnO over graphene along the zigzag edges. Sequential time-frame TEM images showing growth of epitaxial ZnO in from frame 1 to frame 7. The scale bar corresponds to 1 nm. y–z) Clusters of CsI on graphene. y) The HRTEM image of most commonly occurring clusters of CsI on single-layer graphene. The insets show the 4-, 6-, and 7-feature clusters, as indicated by the green, red, and blue boxes, respectively. z) shows the overview of 2D CsI crystal on bilayer. Both monolayer and bilayer crystals were observed, as it can be seen from the contrast and marked by the green and yellow arrows respectively. Insets show the magnified atomic resolution image of the single-layer and double crystals, as marked in green and yellow boxes, respectively. a–o) Reproduced with permission. \cite{66} Copyright 2018, American Chemical Society. p–s) Reproduced with permission. \cite{131} Copyright 2009, American Chemical Society. t–w) Reproduced with permission. \cite{142} Copyright 2015, American Chemical Society. x) Reproduced with permission. \cite{143} Copyright 2016, American Chemical Society. y–z) Reproduced with permission. \cite{144} Copyright 2020, American Chemical Society.
monolayer ZnO layer can grow heteroepitaxially, atom by atom, when irradiated with e-beam, as shown in Figure 8x. They speculated that formation energies of ZnO, especially at the O- and Zn-terminated edges, decreases rapidly as the growth step increases. This study could lead to a new class of 2D lateral heterostructures, fabricated with high spatial control over epitaxial growth.

3.2. Functionality and Applications

3.2.1. Free Standing Monolayers

Graphene is known to have defects. Nanopores can be created in graphene either during synthesis or when the defects congregate under the exposure to e-beam. These nanopores show some stability and the smaller ones have been reported to be often stabilized by lightweight atoms such as H and Si that act as a bridge between the dangling bonds around the perimeter of the hole. The interesting phenomenon to note in graphene substrate is that even these defective regions in graphene can contribute to the formation of monolayer atomic thick metals or metal oxides. Zhao et al. showed that Fe atoms get diffused on the surface of graphene to the edges and then self-assemble in a graphene nanopore to form freestanding crystalline single-atom-thick layer with either body-centered cubic (BCC) packing or HCP structure, as shown in Figure 9a–g. The diffusion of Fe on graphene membrane can be said to be largely electron driven. Even metal oxide showed similar phenomenon when introduced to graphene system. When driven by the e-beam, small clusters of CuO nanosheets with a square Cu sublattice. The sheets were formed on graphene as well as suspended in a graphene nanopore, as shown in Figure 9l–n. The authors used theoretical calculations to predict that standing CuO monolayer has indirect bandgap of around 3 eV, which is significantly higher than bulk CuO whose indirect bandgap is of around 1.5 eV. Using precisely controlled e-beam to sputter atoms, if half of the O atoms are further removed from the CuO crystal cells, then CuO may transform into freestanding monolayer Cu2O, which is another stable material but with a direct bandgap. Manipulation and tuning of the structural morphologies of such materials holds promises in applications in the area of optoelectronics. Similar findings of membrane formation, restructuring, and atomic diffusion was found for freestanding monolayer and bilayers of ZnO on graphene.

Structure of planar ZnO had been predicted theoretically and studied via STEM on a surface but the study of freestanding monolayer ZnO via TEM sheds light upon its actual lattice parameter, structure, and stability. Similar to what has been observed for Fe and CuO membranes, the ZnO sheet was also found to be relatively stable under electron irradiation, as shown in Figure 9o–v. Ta et al. also showed that when ZnO is introduced to graphene, it can effuse out of the material through small rupture to then also form freestanding monolayers. Recently, Ta et al. demonstrated the first 2D single-atom-thick freestanding pure crystalline Cr membranes in graphene pores. They showed that with the help of e-beam, they were able to eject C and O atoms from an amorphous composite film of Cr—O—C supported on monolayer graphene film. The freestanding monolayer was formed in nanopores of diameter 1–2 nm. These results confirmed the existence of freestanding 2D metal membranes and can pave way for studying the novel class of elemental 2D metals. Theoretical works conducted on the freestanding 2D metal monolayers in graphene nanopores predict that the metal monolayers can be stable in area up to ∼8 nm² areas and serve as a platform for a number of applications such as catalysis, bioimaging, photovoltaics, sensing, etc. Further investigations in this area would pave way for formations of potentially novel 2D structures within the graphene membrane for spintronic applications.

3.2.2. Electrocatalyst

In the emerging field of heterogeneous catalysis, where the phase of the catalysts are different to the phase of the reactant or products, the structural parameters of a catalyst play a huge role which directly affect its catalytic performance. These parameters include the size and shape of the catalyst, its chemical composition and accurate atomic structure, bonding between the atoms, charge transfer within and outside the catalyst, etc. With the advent of HAADF-STEM, it has become feasible to easily and directly image catalytic nanometal particles or clusters, which gives a lot of information about their size, structure, composition, numbers and distribution on the surface. As most of the catalyst nanoparticles consist of heavy metal atoms, HAADF-STEM is especially beneficial to carry out their characterization as the image intensity is proportional to the atomic number square (Z²) leading to bright Z-contrast. Moreover, STEM also provides precise mapping of the positions of the atoms on the substrate, especially if the substrate is graphene, whose thinness allows feasible mass measurement of individual atoms or clusters.

Another important parameter affecting the catalytic performance is its interaction with the support material and this is yet another avenue where the use of graphene can yield interesting results. The large surface area, chemical stability, and electrical conductivity of graphene also makes it an attractive substrate for single-atom electrocatalysts. Nanocatalysts, whose electrochemical activity is closely connected to the substrate interaction, can be studied easily on top of graphene and the catalytic reactions can also be studied using in situ STEM observations on top of thermally stable graphene.

Some studies have also indicated direct graphene interaction with nanocatalysts. Miramontes et al. used STEM to characterize and configure atomic clusters of rhenium, up to 2–13 atoms per unit and studied their octahedral and tetrahedral morphologies to predict more complex morphologies on top of graphene. With the use of HAADF-STEM, it was possible to differentiate between spots corresponding to single or a column of atoms in a low-atomicity cluster as well as to determine the number of atoms in the cluster with great accuracy as the intensities differ under this imaging mode. Interestingly, they showed that the interacting atoms in the Re clusters had high positive charges, whereas the atoms at the top of the clusters had small negative charges. The study calculated that graphene had periodically altered positive and small negative charges but negative charge was prominent, that compensated for the overall positive
Figure 9. a–h) TEM images of freestanding Fe monolayers on graphene nanopores taken at 80 keV acceleration voltage. Graphene nanopores are highlighted in (a), BCC type monolayer Fe packing in (b), HCP-type monolayer Fe packing in (c), clusters of atoms embedded in graphene in (d), individual Fe atoms at the edges in (e), freestanding Fe monolayers suspended across graphene nanopore in (f–g) and schematic atomic model of monolayer suspension of Fe atoms in graphene (h). All scale bars correspond to 1 nm. l–n) CuO monolayers on graphene surface. ADF-STEM image of CuO clusters at the step edge of graphene (l) restructuring into monolayer CuO sheets (j) and CuO cluster at the edge of a graphene nanopore (k) diffusing into the nanopore to form freestanding CuO monolayers in the nanopore (l). Schematic atomic model with top and side view showing the formation process of CuO monolayer on top of graphene (m) and a theoretical model of a freestanding CuO sheet. The blue, red, and gray balls correspond to copper, oxygen, and carbon, respectively. Scale bars correspond to 1 nm. o–v) Freestanding mono- and bilayer ZnO membranes. TEM image (o) and its image simulation (p) of mono- and bilayer ZnO regions. Inset in (p) shows the quartzite structure. Normalized intensity profiles (q–r) for the experimental image and its simulation, respectively, showing bi-monolayer (green/pink line), bilayer in vacuum (red/dark blue line), monolayer in vacuum (black/yellow line), and wurtzite structure in the inset (light blue/light blue line), respectively. Top view and 3D view of DFT simulated freestanding monolayer (s–t) and bilayer (u–v) ZnO growth on graphene. All scale bars correspond to 2 nm. a–h) Reproduced with permission.[148] Copyright 2014, AAAS. l–n) Reproduced with permission.[120] Copyright 2016, IOP Publishing Ltd. o–v) Reproduced with permission.[149] Copyright 2016, American Chemical Society.
charge of the Re adsorbed clusters. Therefore, due to the net positive charge of the clusters, the authors indicated that higher catalytic effect could be expected when these Re clusters where adsorbed on graphene, as compared with individual atoms.\textsuperscript{[163]} Graphene plays an important role in increasing the catalytic effect as the hybridization of d-states of Re with the states of graphene generated unoccupied d-states close and above the Fermi level. Similar study by Bulusheva et al.\textsuperscript{[164]} proposed that pyridine-type nitrogen atoms located at the edge of graphene sheets were essential to stabilizing single atom catalysts on graphene substrate. This not only changed the interactions of the metal species with the graphene support, but also changed the electronic state of the catalysts. The authors showed that the metal atoms anchored to graphene indicated strongly electron-deficient or ionic state, like they exhibit in metal complexes. This is consistent with the electronic state changes in Re atom clusters on graphene, as discussed earlier. These results are very useful to study further control of the chemical activities of nanocatalysts, either as a single atom or in clusters, with the use of graphene. Numerous other studies of single atom metal and clusters catalysts supported on graphene substrate have been conducted via HAADF-STEM till date, such as, Re,\textsuperscript{[162]} Pt-group metals,\textsuperscript{[164]} Pt,\textsuperscript{[164]-[166]} Pt clusters,\textsuperscript{[167]} Pd,\textsuperscript{[168]} Pt/Pd particles,\textsuperscript{[169]} Ru,\textsuperscript{[170]} on their synthesis, characterization, and applications.

3.2.3. Liquid Cells

Liquid-cell TEM has historically been very important in carrying out ground-breaking research in understanding the kinetically dominated nanocrystal reactions\textsuperscript{[171]} and their growth, nucleation and other dynamic activities,\textsuperscript{[172,173]} as it allows for the direct imaging of the structural transformations at single atomic level in a liquid.\textsuperscript{[174,175]} It has also been used for studying numerous other dynamic activities of nanomaterials in hydrated state including, and not limited to, their morphology, structural evolution, intermediate self-assembly stages, etc.\textsuperscript{[176]}

Different architectures have been used till date to fabricate liquid cells for microscopy purposes.\textsuperscript{[177]} Among all the approaches, the use of graphene as a window material for the fabrication of liquid cells, also known as graphene liquid cells (GLCs), has been used extensively and has consistently been proved to be a better candidate than conventional windows for high-resolution imaging of beam-sensitive specimens.\textsuperscript{[102]} The architecture of such cells comprises only layers of graphene to encapsulate liquid specimens. Such architectures can be built by either encapsulating the liquid between two graphene layers that is suspended across a thin membrane (Figure 10a) or by enclosing holes in the membrane containing liquid by graphene on both side (Figure 10b). In addition to the properties of graphene that make it an excellent substrate for TEM, its highly impermeable property does not even allow small helium atoms to permeate.\textsuperscript{[178]}

Moreover, the graphene sheets are highly inert and flexible and show strong van der Waals interaction to tightly wrap around the liquid inside\textsuperscript{[179,180]} while at the same time withstanding the massive amount of pressure difference within and outside the liquid cell.\textsuperscript{[188]} Therefore, the use of graphene also provides a wider variety of choice in terms of using different kinds of liquid while at the same time providing the ability to carry out microscopy using the usual TEM holder. The exceptional spatial resolution provided using the ultrathin material opens doors for studying nanostructures dynamics at an atomic scale in a liquid environment.\textsuperscript{[179]} For instance, Hauwiller et al.\textsuperscript{[171]} recently showed exciting etching phenomenon on gold nanocrystals in GLCs, shedding light upon the presence of high-energy intermediate shaped in a kinetically controlled reaction which was not known previously. As a result, there has been a massive progress in this field of GLCs in the past few years, leading to understanding toward the dynamic behaviors of nanomaterials inside a liquid.\textsuperscript{[181]}

GLCs have also facilitated exciting research and findings in the field of nanoscience, such as crystallization and in situ crystal growth,\textsuperscript{[182]} nucleation and shape formation of crystals,\textsuperscript{[183]} study of molecular structure of water and ice,\textsuperscript{[184]} lithium transfer in battery materials,\textsuperscript{[185]} high-resolution study of beam-sensitive biological cells,\textsuperscript{[186]} study of intermediate states during the self-assembly of DNA double helices,\textsuperscript{[187]} and exceptional spatial resolution study of nanocrystal etching on a single particle level.\textsuperscript{[188]}

One of the biggest advantages of graphene-based liquid imaging is that it allows the highest possible resolution, whereas at the same time providing a flexible covering to the liquid inside. It has been especially shown to have brought deep understanding in the area of beam-sensitive biological specimens\textsuperscript{[189]} and atomic scale reactions occurring in new energy materials through the study of in situ TEM of anodes, cathodes, and lithium transfer.\textsuperscript{[190]}

Graphene cells combined with emerging microscopy techniques have the potential to study new science and shed light on the dynamics of nanostructures at the lowest possible atomic scale.
4. Conclusion

Graphene has been used as a versatile substrate for studying other nanomaterials under STEM. Moreover, the facile and preparation of large-area suspended graphene on TEM grids, makes it easy to handle in the lab and reproduce the experiments. However, it is important to note the major impact that the amorphous carbon layer on top of the graphene has, on enabling the study of nanoparticles. Graphene itself is known to have small energy barrier for migration of nanomaterials, and it is the amorphous carbon, impurities, and defects in graphene that makes it possible to study other atoms on its surface. With the advent of new materials and techniques, it is a possibility that in the coming decade, we will be able to obtain pristine graphene without any impurities or defects. However, this can have a possible downside for studying materials on its surface since they would not be stable and migrate to the edges. The role of impurities on graphene should be kept in mind while using this material as a substrate for microscopy. Another important point to keep in mind is that graphene has a big influence on the structure and phase transformation of the material that is being studied on its surface, as shown with the study of PdSe$_2$[77] and PbI$_2$.[83] Therefore, it is of immense importance to decouple the role of graphene from the observed results so as to understand the intrinsic behavior of the material that is being studied. In the next decade, I believe that we will be better able to understand the electronic and structural influence of graphene on different materials, especially in the presence of electron beam and use the information to understand the intrinsic properties of other materials, as well as make novel hybrid materials with graphene. It is astonishing how much has already been achieved in the field which is only a few years old. One can anticipate many new exciting e-beam studies, especially with the increased use of STEM, without the use of any other external stimuli, would ultimately allow studying and engineering of new heterostructures with the use of graphene. One of the biggest avenues of research to watch out for is the GLCs. GLC is a particularly good example that truly reflects the uniqueness of graphene film and opens the possibility to explore materials that have not been studied previously due to lack of experimental capabilities.

Experiments have shown exciting results on the dynamics, chemistry, and behavior of nanomaterials in TEM facilitated using graphene as a substrate. The work carried out in this field also provides new avenues of research in the area of graphene-hybrid materials and their interactions with the e-beam, especially using ADF-STEM. TEM can also act as a tool to manipulate structures of nanomaterials. That being said, it is a challenge to control the spatial and precise manipulation of atoms via TEM because of the complex interaction between the e-beam and the materials. However, STEM mode of imaging does make it feasible to use controlled electron beam irradiation which can potentially be used for single-atom manipulation.[191] Electron beam-driven chemistry in and around graphene demonstrates how it can also serve as a platform for growth of new nanocrystals and in-plane epitaxial alignment of nanomaterials. This is one of the areas where graphene has actually proved to have immediate applications. However, electron beam induced displacement for single-layer graphene should be kept in mind while doing these experiments, as has been clearly shown by Meyer et al.[19] Their work concluded that although defect free imaging of single-layer graphene is easily possible at an accelerating voltage of 80 keV, 100 keV TEM images already not represent the original configuration of a sample. Therefore, in practice, such imaging of graphene heterostructures should not take place in a high-voltage and high-dose conditions and ideally take place below 80 keV, or otherwise the knock-on damage on graphene should be separately considered during the analysis. The combined use of low-voltage electron microscopy on light elements like Li with graphene encapsulation,[192] has been shown to reduce knock-on damage as well as facilitate successful in situ electrical measurements inside the microscope. We can hope that the use of graphene would further develop other directions in microscopy, especially various in situ techniques.

Acknowledgements

S.S. thanks the joint committee of Linacre College and MPLS division of the University of Oxford for financial support through the Women in Science Scholarship and EPSRC Studentship.

Conflict of Interest

The authors declare no conflict of interest.

Keywords

2D materials, electron transparent support, graphene, transmission electron microscopy

Received: July 24, 2020
Revised: November 13, 2020
Published online: January 15, 2021

[1] L. Gross, F. Mohn, N. Moll, G. Meyer, R. Ebel, W. M. Abdel-Mageed, M. Jaspers, Nat. Chem. 2010, 2, 821.
[2] A. Riss, A. P. Paz, S. Wickenburg, H.-Z. Tsai, D. G. De Oteyza, A. J. Bradley, M. M. Ugeda, P. Gorman, H. S. Jung, M. F. Crommie, A. Rubio, F. R. Fischer, Nat. Chem. 2016, 8, 678.
[3] H. Zhou, J. Liu, S. Du, L. Zhang, G. Li, Y. Zhang, B. Z. Tang, H.-J. Gao, J. Am. Chem. Soc. 2014, 136, 5567.
[4] M. Di Giovannantonio, M. El Garah, J. Lipton-Duffin, V. Meunier, L. Cardenas, Y. Fagot Revurat, A. Cossaro, A. Verdini, D. F. Perepichka, F. Rosei, G. Contini, ACS Nano 2013, 7, 8190.
[5] Q. Sun, C. Zhang, Z. Li, H. Kong, Q. Tan, A. Hu, W. Xu, J. Am. Chem. Soc. 2013, 135, 8448.
[6] J. Björk, Y.-Q. Zhang, F. Klappenberger, J. V. Barth, S. Stafström, J. Phys. Chem. C 2014, 118, 3181.
[7] L. Gross, B. Schuler, N. Pavliček, S. Fatayer, Z. Majzik, N. Moll, D. Peña, G. Meyer, Angew. Chem., Int. Ed. 2018, 57, 3888.
[8] E. I. Altman, M. Z. Baykara, U. D. Schwarz, Acc. Chem. Res. 2015, 48, 2640.
[9] A. Bachmatiuk, J. Zhao, S. M. Gorantla, I. G. Gonzalez Martinez, J. Wiedermann, C. Lee, J. Eckert, M. H. Rummeli, Small 2015, 11, 515.
[10] A. I. Kirkland, S. L. Chang, J. L. Hutchison, in Springuer Handbook of Microscopy, Springer, Cham 2019.
[11] A. K. Geim, K. S. Novoselov, Nat. Mater. 2007, 6, 133.
[12] K. S. Novoselov, A. K. Geim, S. V. Morozov, D. Jiang, Y. Zhang, S. V. Dubonos, I. V. Grigorieva, A. A. Firsov, Science 2004, 306, 666.
Sapna Sinha is a postdoctoral researcher in the Department of Materials at the University of Oxford. Her research involves synthesis, processing, and application of nanomaterials for energy and electronics. Recent work includes using graphene as an electron transparent support for direct imaging of small molecules and nanomaterials using transmission electron microscopy.

Jamie Warner is the Hayden Centennial professor in the Department of Mechanical Engineering and the Director of Electron Microscopy in the Texas Materials Institute, The University of Texas at Austin. His research focuses on structure of small nanomaterials using low voltage transmission electron microscopy and developing methods to image beam-sensitive crystal structures.