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TRENDS

Peptide interfaces with graphene: an emerging intersection of analytical chemistry, theory, and materials

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Abstract Because noncovalent interface functionalization is frequently required in graphene-based devices, biomolecular self-assembly has begun to emerge as a route for controlling substrate electronic structure or binding specificity for soluble analytes. The remarkable diversity of structures that arise in biological self-assembly hints at the possibility of equally diverse and well-controlled surface chemistry at graphene interfaces. However, predicting and analyzing adsorbed monolayer structures at such interfaces raises substantial experimental and theoretical challenges. In contrast with the relatively well-developed monolayer chemistry and characterization methods applied at coinage metal surfaces, monolayers on graphene are both less robust and more structurally complex, levying more stringent requirements on characterization techniques. Theory presents opportunities to understand early binding events that lay the groundwork for full monolayer structure. However, predicting interactions between complex biomolecules, solvent, and substrate is necessitating a suite of new force fields and algorithms to assess likely binding configurations, solvent effects, and modulations to substrate electronic properties. This article briefly discusses emerging analytical and theoretical methods used to develop a rigorous chemical understanding of the self-assembly of peptide–graphene interfaces and prospects for future advances in the field.

Keywords Graphene · Peptide · Scanning tunneling microscopy · Atomic force microscopy · Polarization modulation IR reflection–absorption spectroscopy · Quartz crystal microgravimetry

Introduction

Since its discovery in 2004 [1], graphene has been studied extensively because of its exceptional properties [2–4], including high room temperature conductivity [1], impressive mechanical strength [5], half-integer quantum Hall effect [6], and massless Dirac fermion transport capabilities [7]. Strategic functionalization of the graphene surface can modulate its interactions with analytes [8], its solubility [9, 10], and its local bandgap [11]; functionalization is particularly critical for biological applications, because the hydrophobic graphene surface can otherwise cause proteins to denature [12]. Many applications take advantage of noncovalent modification strategies [13–15] to preserve the high conductivity and intrinsic strength of the graphene sheet [16–25].

Interfaces between graphene and polypeptides or proteins have been of particular interest because of the chemical diversity that can be engineered into the interface, mirroring the diversity of biological structure and function. Even fairly simple interfaces can be useful: graphene and its derivatives have catalyzed hydrolysis of proteins [26], formed nanowire hybrids with polyalanine [27], and acted as templates for protein and peptide organization via noncovalent binding motifs [28, 29]. Taking advantage of graphene's electron transport properties and susceptibility to molecular doping [30] also permits detection of analyte binding from solution, even at extremely low

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concentrations [31]. The ability to arbitrarily design peptide–graphene interfaces with molecular precision would open further possibilities ranging from molecular logic devices [32] to biocatalytic reactor surfaces similar to enzymes [33].

However, the structural and chemical diversity of the interface also creates a set of critical analytical and predictive challenges (Fig. 1). When a peptide adsorbs to graphene, one face interacts with the graphene substrate (important for adsorption stability and/or electronic doping) and one face is exposed to the solvent (important for analyte binding, solubilization, or coupling to create extended materials). Because noncovalent adsorption depends on a delicate balance of molecule–substrate, molecule–molecule, and molecule–solvent interactions [34], a single peptide can have many binding modes. Creation of well-defined interfaces requires the ability to predict peptide adsorption geometries on graphene and to analyze details of peptide binding, including ordering and orientation. The analytical challenges here *also* mirror those in biology—those related to protein folding [35]. Just as with protein folding, assembly involves hydrophilic–hydrophobic interfaces, a vast conformational space, and many local energy minima. At the same time, graphene also makes fundamental changes to the characterization problem, because of its 2D structure, conductivity, and strong optical absorbance.

The ability to achieve both predictive and analytical goals lies near the current limits of theory and experiment. This article first discusses selected examples of bioanalytical applications to provide context for the utility and general structures of peptide–graphene interfaces. Next, we discuss analytical techniques, first those used predominantly to characterize the graphene component of the interface, then we highlight a subset of techniques that provide more detailed information about peptide adsorption and ordering. Recent advances in modeling peptide–graphene interfaces are also examined, with discussion of the trade-offs that are frequently required in approximating the behavior of the peptide, the solvent, and the substrate. Finally, we provide a brief forward-looking perspective on opportunities for development of experimental and theoretical methods in this area. Although both graphene and graphene oxide have been widely used as substrates for the assembly of peptides, here we largely focus on pristine graphene and graphitic (e.g., highly oriented pyrolytic graphite, HOPG) interfaces, which are more straightforward substrates for detailed characterization and modeling of the molecule–substrate and molecule–molecule interactions that drive assembly. Insights from pristine graphitic materials can ultimately be used to improve understanding of other graphene derivatives.

Context: applications of peptide–graphene interfaces

Applications of peptide–graphene interfaces may utilize the electronic, optical, and/or mechanical properties of the graphene substrate, which arise from its regular lattice structure. These are combined with the diverse and powerful chemical specificity available from peptides to afford molecular recognition, solubility, spatial ordering, or other properties [36].

For instance, graphene-based sensing applications frequently leverage either conductivity changes produced when an analyte binds to the interface and creates local electronic doping, or fluorescence quenching effects. Early sensors based on nanowires and nanotubes exhibited excellent sensitivity but limited specificity [37]; engineering a peptide monolayer substantially increases the analytical discriminating power of graphene interfaces for sensing. Mannoor et al. [38] designed a wireless bio-interfaced sensor, based on bifunctional peptides designed to both bind graphene and specifically detect desired bacterial species (Fig. 2a). On changes in electronic conductivity (e.g., through a binding event), an electromagnetic signal would be induced and wirelessly transmitted by a gold coil patterned on the graphene. To specifically detect bacteria, a graphene-binding peptide (GBP) was covalently linked via a triglycine sequence to antimicrobial peptide odorranin-HP, which shows specificity toward disease-relevant bacteria: *Escherichia coli*, *Helicobacter pylori*, and *Staphylococcus aureus* [39]. The device operated successfully in complex mixtures, detecting *S. aureus* content as low as one bacterium per microliter of blood in an intravenous bag, and *H. pylori* binding to a bovine tooth, with a lower detection limit of about 100 cells [40].

Graphene's fluorescence quenching properties can also be utilized in the design of biosensing devices. Frequently such applications use graphene oxide, because of its increased solubility [37]. For example, Zhang et al. [41] designed a protease monitoring device utilizing fluorescence resonance energy transfer with a graphene oxide–peptide interface (Fig. 2b). When a fluorescein isothiocyanate (FITC)-labeled thrombin-recognizing peptide (sequence KCALNNGSGdFPRGRAK) was mixed with graphene oxide, the FITC fluorescence was quenched as the fluorophore was brought near the graphene surface. Thrombin, a serine protease important in platelet activation, works by cleaving the Arg–Gly bond, which in this case released the FITC tag, restoring its fluorescence. Here, the sensor was able to detect thrombin activity at peptide concentrations as low as 2 nM.

Thus, both the electronic and the optical properties of biomolecule–graphene interfaces can be used in biochemical assays. However, these applications require specific adsorption configurations to ensure the availability of one segment of the peptide to a solvated binding partner (e.g., thrombin), and simultaneously, the strong binding of another segment to the graphene interface. Similar requirements are levied in other applications, such as the development of hybrid materials, in which the peptide must either passivate or electronically modulate the substrate, while also providing solubility and/or molecular recognition to couple elements of the material [42].

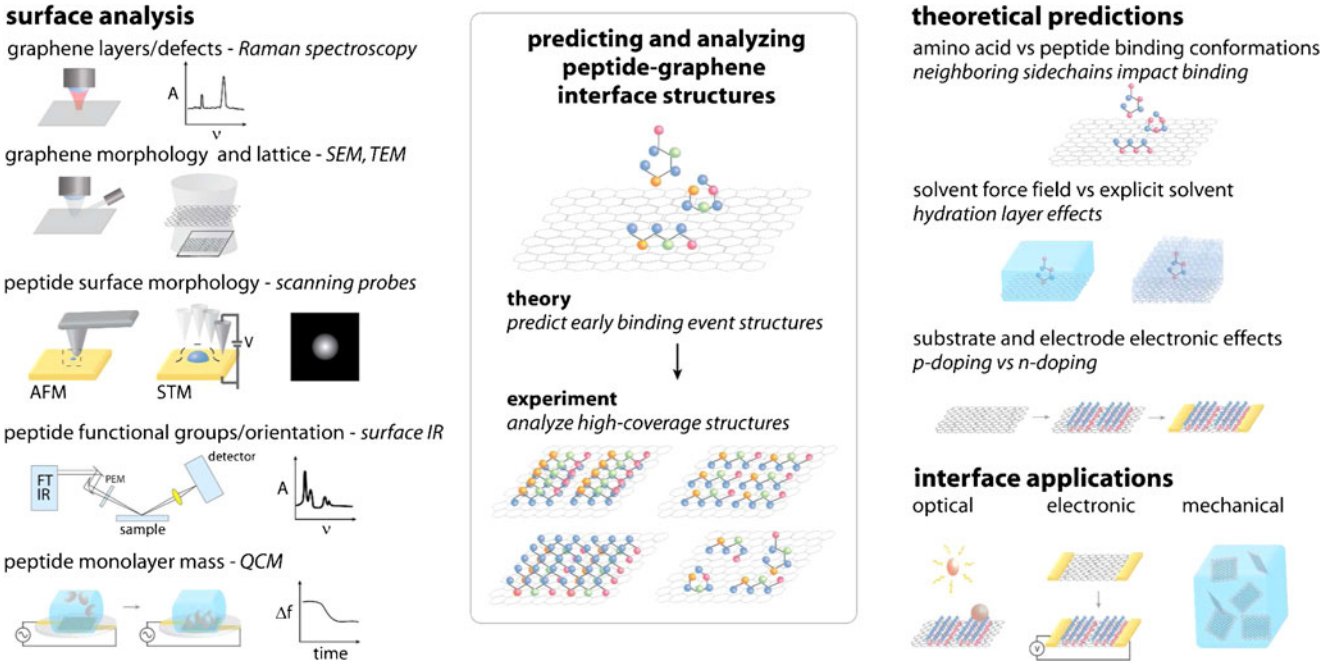

Fig. 1 The development, characterization, and utilization of peptide–graphene interfaces represents an emerging frontier for analytical chemistry and theory. *AFM* atomic force microscopy, *FTIR* Fourier transform IR, *PEM* photoelastic modulator, *QCM* quartz crystal microgravimetry, *SEM* scanning electron microscopy, *STM* scanning tunneling microscopy, *TEM* transmission electron microscopy

Analytical techniques applied to peptide–graphene interfaces

Continued development of peptide–graphene interfaces [20–22] will benefit from detailed analysis of interface structure. This is analogous to the impact interface-sensitive analytical techniques have had on progress in the field of alkanethiol self-assembled monolayers on coinage metal and other surfaces [43–45]. Surface IR spectroscopy [46], X-ray photoelectron spectroscopy [47], and scanning probes—for example, atomic force microscopy (AFM) and scanning tunneling microscopy (STM) [48–50]—have elucidated molecular tilt angles, binding energies, and lattice structures of such self-assembled monolayers. A detailed understanding of structural aspects of self-assembled monolayers has opened up new applications in the field of nanoscience [43, 51], ranging from bio-inspired mineralization [52] to molecular electronics [53]. The noncovalent monolayer structures formed by peptides on graphene necessitate certain differences in characterization methods to establish ordering and orientation. For instance, whereas X-ray photoelectron spectroscopy is routinely used to establish Au–S bond formation in alkanethiol monolayers [43] (a starting point for understanding molecular orientation), peptide–graphene interfaces lack this type of spectroscopic signature.

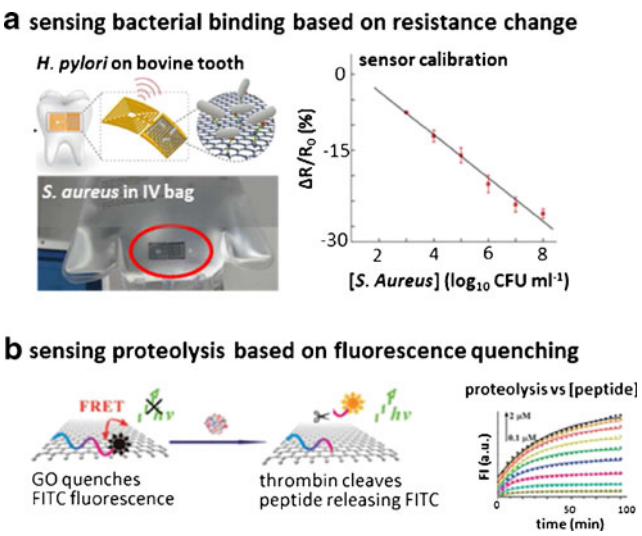

Fig. 2 Analytical devices based on biomolecule–graphene interfaces. **a** Functionalized graphene interface conductivity changes in response to bacterial binding. **b** A graphene oxide (*GO*)–peptide sensor monitors protease activity on the basis of an increase in fluorescence as fluorescein isothiocyanate (*FITC*) is released after peptide cleavage by thrombin. *CFU* colony-forming units, *Fl* fluorescence, *FRET* fluorescence resonance energy transfer, *IV* intravenous. (Adapted with permission from [38, 41])

A number of interface-sensitive techniques are useful for the characterization of peptide–graphene assemblies at the material scale (e.g., structure of a graphene sheet or morphology of a peptide aggregate on the sheet). For instance, Raman spectroscopy is routinely used to analyze both the number of graphene layers (on the basis of the intensity ratio between the G band peak at approximately 1586 cm^{-1} and the 2D peak at approximately 2695 cm^{-1}) and the presence of graphene defects (on the basis of the intensity of the D band peak at

approximately 1350 cm^{-1}) [54]. For instance, in preparing peptide–graphene hybrid materials, Lerner et al. [55] used Raman spectroscopy to evaluate changes in the graphene sheet structure after treatment with diazonium salts and before mixing with peptides (Fig. 3a). Although the technique is informative in analysis of graphene structure, low Raman scattering cross sections of most organic molecules typically preclude spectroscopy of peptide monolayers. Scanning electron microscopy, with a typical spatial resolution of 5–10 nm [56], is useful in assessing the 3D morphologies of graphene sheets; Fig. 3b shows the technique used to visualize the rolled geometry of a graphene sheet enveloping a peptide fibril [27]. Transmission electron microscopy (TEM) has spatial resolving power adequate to image sub-nanometer atomic lattices in nanoscopic metals and semiconductors [57] and to observe morphologies of large supramolecular organic structures such as amyloid fibrils interfaced with graphene (Fig. 3c) [58]. Visualizing organic materials frequently requires staining with contrast agents such as uranyl acetate, since electron scattering is proportional to atomic number. This raises challenges in detecting structure in heterogeneous monolayers of organic material (e.g., peptides), although the more regular structures of graphene and graphene oxide can be resolved with aberration-corrected high-resolution instrumentation. For instance, Fig. 3d shows high-resolution TEM images of a graphene oxide substrate in which the lattice is visible in parts of the layer. To the right in Fig. 3d, a model and simulated image show a ferritin protein with a nanocrystalline ferrihydrite core. The core and its lattice structure are visible in the high-resolution TEM image (bottom of Fig. 3d), whereas the lower-contrast organic protein material is not easily resolved [59]. Continued advances in this instrumentation (e.g., aberration-corrected lenses [57] and graphene liquid cells [60, 61]) may ultimately make such characterization more feasible for thin, heterogeneous organic structures such as peptide monolayers as well [62].

Certain surface analysis techniques, including scanning probes [63], surface IR spectroscopy, and quartz crystal microgravimetry (QCM), have been successfully applied to analyze details of monolayer structure and assembly dynamics in peptide interfaces with layered materials.

Both AFM [64–66] and STM [67, 68] have proven useful in this regard, because of their extremely high spatial resolution (typically 1 nm lateral and 0.1 nm vertical for AFM; less than 0.1 nm lateral and vertical for STM). For instance, Claridge et al. [68] used a combination of AFM and STM to observe structures of small model amyloid peptides forming β sheets at graphitic interfaces. Figure 4a shows AFM images of peptide lamellar structures with a periodicity of approximately 5 nm formed in epitaxy with the hexagonal graphite lattice. STM images (Fig. 4b) resolved individual peptides with a lateral spacing of approximately 0.45 nm characteristic of a β sheet, and textural differences corresponding to repeats of histidine and alanine residues. However, the relatively weak noncovalent adsorption mechanism raises challenges for scanning probes, evident in the ultrahigh vacuum STM image (Fig. 4c), in which the motion of the probe sweeping across the surface results in streaking as some peptides in the β sheet

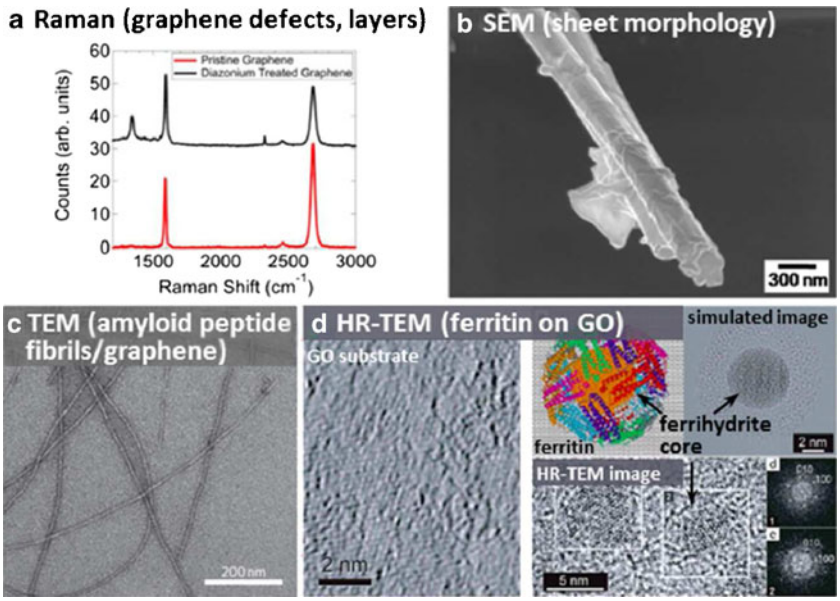

Fig. 3 Analytical techniques applied to graphitic interfaces with biomolecules. **a** Raman spectroscopy used to characterize formation of defects in graphene during preparation of peptide–graphene hybrid materials. **b** Scanning electron microscopy (*SEM*) used to characterize graphene sheet morphology following exposure to peptide nanotubes. **c** Transmission electron microscopy (*TEM*) used to visualize peptide fibril morphology on a graphene support. **d** High-resolution (*HR*) TEM used first to visualize local lattice structure in graphene oxide (*GO*) support, and ferritin proteins, including ferrihydrite nanocrystal core. (Adapted with permission from [27, 55, 58, 59])

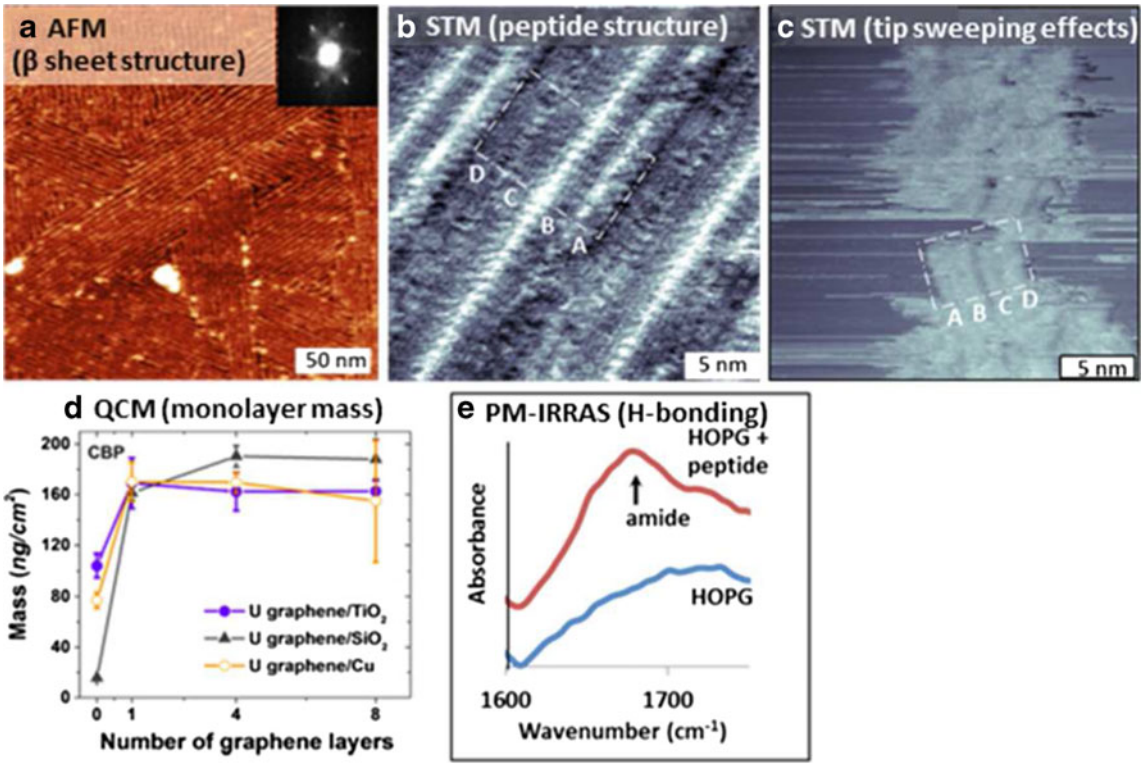

Fig. 4 Analytical techniques applied to graphitic interfaces with biomolecules. **a** Atomic force microscopy (*AFM*) resolves peptide β sheets on highly oriented pyrolytic graphite (*HOPG*). **b** Scanning tunneling microscopy (*STM*) resolves submolecular structure in individual peptides **c** Tip dragging effects are problematic for scanning probes in low-coverage monolayers **d** Quartz crystal microgravimetry (QCM) resolves sub-nanogram changes in interfacial mass as molecules adsorb during monolayer formation. **e** Polarization modulation IR reflection absorption spectroscopy (PM-IRRAS) detects bond vibration shifts due to hydrogen bonding in peptide monolayers. *CBP* carbon-nanotube-binding peptide. (Adapted with permission from [68, 71])

become dislodged. Although scanning probe techniques cannot typically probe fast interfacial dynamic events, AFM imaging has frequently been used to observe self-assembly dynamics of peptide–graphite interfaces in liquids on timescales of minutes to hours, as in the earlier work of Kowalewski and Holtzman [69]. AFM tips can also be functionalized with a molecule of interest (e.g., a biotin tether) and brought in and out of contact with a functional surface (e.g., streptavidin modified) to measure the strength of a binding interaction [70], suggesting the possibility of the use of peptide-modified AFM tips to measure the strength of interactions with a graphene surface.

The kinetics of early binding events during monolayer formation can be probed by means of QCM [72], which detects mass changes as small as 1 ng associated with analytes (including biomolecules) binding at an interface. The typical monolayer mass for an area the size of a commercial QCM sensor (e.g., circular film 2 mm in diameter) is on the order of 10–100 ng, making it possible to probe monolayer assembly with time resolution of approximately 1 s. Kim et al. [71] used this approach to analyze the amount of a GBP that adsorbed on a set of graphene interfaces on the basis of the number of layers (zero to eight) and the support substrate (SiO_2, TiO_2, or Cu) (Fig. 4d). For thicker films of soft materials (e.g., those using antibodies and other large biomolecules, which may have diameters greater than 10 nm), dissipative losses must be accounted for [73], although for nanometer-thick layers such as lying-down monolayers of peptides this is less of an issue.

The chemical environment (and in some cases orientation) of functional groups at a graphene interface can be assessed by use of IR reflection techniques, including attenuated total reflection spectroscopy and IR reflection–absorption spectroscopy (IRRAS) [68, 74]. These techniques can be used to analyze hydrogen bonding and other noncovalent interactions within a monolayer, to monitor the assembly process. Shifts in the amide I band in an IR reflection absorption spectrum provide a readout of peptide secondary structure, with peak positional differences corresponding to α helices, β sheets, and disordered structures (Fig. 4e) [75]. For detailed characterization of monolayer structure, it is also useful to examine the *orientation* of functional groups relative to the substrate, which can be achieved by use of the subset of polarization modulation approaches such as polarization modulation IRRAS. Because ordered bond dipoles preferentially absorb either s- or p-polarized light depending on their orientation (and surface selection rules), the difference spectrum can be used to assess orientational ordering in nanometer-thick films

at interfaces. Although these techniques do not provide spatially resolved chemical information (typical spot sizes may be up to 1 cm^2), they are useful for analyzing monolayers with long-range order to understand which chemical functional groups will be displayed at the solvent and substrate interfaces.

Theory and experiment used in tandem to predict interface characteristics

Whereas analytical techniques such as AFM, STM, and surface IR spectroscopy typically characterize high-coverage (complete or nearly complete) peptide monolayers on graphene, theoretical simulations shed light on early adsorption events during interface assembly. Fast, accurate modeling would streamline predictive design of surfaces to bind arbitrary analytes, or materials with tailored optical or electronic properties. However, because of the complexity of the interface (i.e., large flexible adsorbates, hydrogen-bonding solvent, electronically polarizable substrate), molecular dynamics methods still require various amounts of approximation to reduce computational burden and run time.

Here, we discuss a set of recent theoretical approaches that incorporate different sets of approximations in order to simulate the adsorption of peptides at a solvated graphene interface. For instance, to model the binding of multiple peptides (important in building up a monolayer structure), one approach parameterizes a peptide as a series of residues rather than incorporating the contributions of each atom (which would be more accurate, but also more computationally costly) [76]. Some calculations use explicit water molecules to understand the role water plays in determining which amino acid residues bind most strongly (e.g., due to ordering of water at the hydrophobic interface, or hydrogen bonding to the peptide) [76, 77]. Other calculations use a force field to represent the solvent (less accurate, but also less costly), meaning that the peptide and graphene contributions can then be modeled in more detail. Finally, the electronic polarizability of the graphene substrate almost certainly plays an important role [77, 78]. A number of force fields have been developed to represent graphene with differing levels of accuracy (and expense): AMOEBAPRO is a fairly widely used option that is both accurate and computationally costly; other alternatives such as GRAPPA are more approximate, but also less computationally costly in cases where solvent or adsorbate contributions are of primary importance.

Peptide binding affinities for graphene are important determinants of peptide–graphene interface behavior; calculated values differ depending on how the contributions of the solvent and the substrate are approximated. For instance, Camden et al. [76] used a computationally efficient "four-box" method (Fig. 5a) to calculate binding enthalpies for peptides binding to graphene [79]; the computational efficiency of the approach allowed the inclusion of explicit water molecules. Surprisingly, in these simulations many residues with hydrophilic side chains exhibited greater binding enthalpies than aromatic residues, because of interactions with the relatively dense first hydration layer at the graphene surface. These calculations were performed with the TEAM force field [80], which parameterizes molecules on the basis of molecular fragments rather than atoms to facilitate model construction. Other computational studies using force fields such as AMOEBAPRO in combination with implicit solvent predict that aromatic residues such as tryptophan should exhibit the strongest binding because of π–π stacking [81]. This divergence raises important questions regarding the relative importance of the contributions of solvent and substrate in the assembly process, in particular the role of water ordering at the hydrophobic interface.

The extensive conformational space for complex peptides requires broad sampling to ensure the lowest-energy conformer is found [82]. Parallel tempering (also known as replica exchange) allows multiple conformations to be sampled simultaneously at different temperatures and interchanged to improve the dynamic properties of the simulation [83]. However, the sheer scale of the conformational space of a peptide means that parallel tempering often requires excessive computational resources. Replica exchange with solute tempering reduces the number of replicas required by varying the temperature of only the solute (as opposed to the solute and solvent) between replicas [84]. Hughes and Walsh [77] used this approach in tandem with the relatively inexpensive GRAPPA force field (which models graphene polarization using a rigid rod dipole, and is less rigorous and expensive than multipole AMOEBAPRO), allowing the use of explicit solvent (Fig. 5b). Again in this approach, a new possible driving principle for self-assembly of peptides on graphene emerges with the use of explicit solvent molecules. In addition to large, planar side groups (e.g., arginine, tryptophan, and tyrosine) typically used in graphene-binding motifs, small compact groups (e.g., glycine) also displayed high binding affinity; adsorption brings the functional groups out of dense hydration layers approximately 3 and 6 Å from the surface. Again, this suggests that a detailed understanding of solvent behavior may be important in the prediction of noncovalent interface structures

In graphene, the sheet edges and basal plane have different chemical characteristics, which can be exploited in the development of peptide–graphene interfaces, making it important to accurately model the substrate. This possibility has been explored both experimentally and theoretically, because it is experimentally straightforward to distinguish between step edge and basal plane adsorption with use of AFM. Experimentally, McAlpine and coworkers [64, 85] have leveraged combinatorial phage display libraries to engineer peptides that

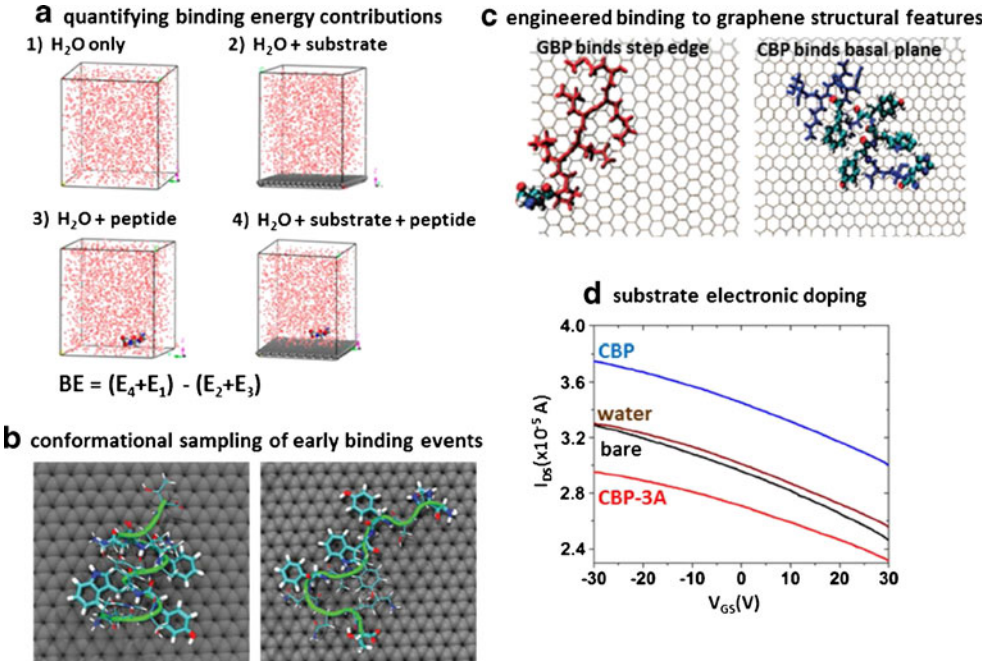

Fig. 5 Modeling of early binding events. **a** Four-box model quantifies solvent, substrate, and peptide contributions to binding enthalpy (*BE*). **b** Replica exchange allows broad conformational sampling to ensure the lowest-energy structure is found. **c** Peptides can be engineered to bind either graphene step edges or graphene basal plane. **d** Theory can be used to predict graphene electronic doping by peptides. *CBP* carbon-nanotube-binding peptide, *GBP* graphene-binding peptide. (Adapted with permission from [76, 78, 85, 86])

not only bind specifically to graphitic interfaces but also exhibit preferences for either step edges or the basal plane (Fig. 5c). For instance, a phage-selected GBP (sequence EPLQLKM) displayed affinity toward HOPG step edges, whereas a previously engineered carbon-nanotube-binding peptide (CBP) (sequence HSSYWYAFNNKT) bound uniformly across the HOPG surface. Molecular dynamics simulations in explicit water indicated that the GBP is attracted to the slightly positive step edges through its negatively charged glutamate residue. Conversely, the CBP maximizes π–π offstacking interactions between its aromatic groups and the graphene surface. In these calculations, approximations were made regarding the peptide: interactions of individual peptides with a graphene sheet were first modeled by all-atom simulations; these results were used to normalize parameters for residue–graphene coarse grain interactions [87, 88] to reduce computational complexity for larger models over longer time frames. Simulations performed with this approach were able to capture the greater basal plane binding potential of CBP versus GBP, as well as the critical importance of the residues YWY in anchoring CBP to the graphene basal plane. Here, the ability to combine experiment with theory helps ensure appropriate levels of approximation are used in the simulation.

In some cases, the goal of creating a non-covalently modified graphene interface is to create local electronic doping (Fig. 5d) [78, 89], making it especially important to accurately model the substrate. Akdim et al. [78] tested peptide doping effects in graphene field effect transistors using both simulations and experiment [85]. The simulations used the AMOEBAPRO force field [90] and implicit solvent. The electron transport properties of the peptide-functionalized graphene were then modeled with use of nonequilibrium Green's functions [91] and density functional tight binding [92], a semiempirical method that allows calculation of the density of states in an extended system. Interestingly, their calculations indicated that p-doping can arise *either* from π stacking with aromatic side chains *or* from interactions with the peptide backbone near residues with small side chains (e.g., alanine), suggesting the possibility of an alternative class of peptide doping motifs. However, whereas the experimental results demonstrated a large p-doping effect for CBP, a small n-doping effect was observed for the alanine peptide. Such divergence could arise from approximations made in the simulation or experimentally from the presence of graphene defects or electronic effects caused by the introduction of metal electrodes. This highlights the need for both improved experimental techniques to assess detailed interfacial structure directly and improved theoretical methods to treat the presence of features observed in real device architectures.

Outlook

Interfaces between layered materials and biomolecules, such as the peptide–graphene interface, have the potential to create fundamentally new types of surface chemistry with applications ranging from sensing to nanoscale electronics to hybrid functional materials. However, complex interactions between biomolecules, solvent, and substrate can result in a variety of adsorption conformations, impacting both substrate electronic structure and solvent interface chemistry in ways that are not currently well predicted. Conversely, this means that a rich

variety of interface structures (both chemical and electronic) will become available if predictive control *can* be developed through a coupling of theory and experiment. A few key issues will likely shape development of this area.

The hydrophobic–hydrophilic interface dynamics important in assembly of biomolecules on graphene present key opportunities for contributions from theory. However, noncovalent interactions are difficult to capture accurately in energy minimizations, and understanding early stages of assembly at hydrated graphene interfaces requires quantification of contributions from both ordered water layers in the nanometer nearest the hydrophobic surface and the electronic polarization of the substrate. Therefore, it is likely that the most successful strategies will develop experimental methods to assess common enthalpic and entropic contributions to assembly and use these known values to reduce simulation complexity. Polarized optical measurement methods such as polarization modulation IRRAS and polarized nonlinear optical spectroscopies that have the potential to resolve bond orientations and vibrational energy shifts at an interface are thus especially promising in this regard.

In the comparison of theory with experimental results, another critical challenge is the imperfection of real interfaces. Although graphene and graphene derivatives are now widely available commercially, variations in manufacturing and transfer procedures can result in batch-to-batch variations that become important in the assembly and characterization of peptide–graphene monolayers. Additionally, recent experiments indicate that in the 24 h following synthesis or thermal annealing to produce a clean graphene interface, adsorption of adventitious contaminants from the laboratory atmosphere substantially changes the surface chemistry [93]. Thus, the capability to not only prepare clean interfaces but also to routinely and quickly assess the presence of non-covalently adsorbed contaminants will become key to successful interface development.

Finally, new experimental techniques that simultaneously offer single-molecule spatial resolution and chemical information have the potential to resolve adsorption geometries and interface chemistry directly. For instance, force-curve-based and molecular-recognition-based AFM measurements can resolve certain types of molecular interactions on a substrate, and STM measurements based on microwave-frequency bias modulation and inelastic tunneling can also be used to resolve the presence of key functional groups [63].

A rigorous understanding of design principles for peptide–graphene interfaces can ultimately be expected to open new routes for not only in vitro sensing and electronics but also for establishment of in vivo interfaces with layered materials. Such applications will allow the exceptional mechanical, optical, and electronic properties of layered materials to be intimately mixed with the diverse and powerful chemistry that emerges from noncovalent interactions in biology.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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