Fabrication and biosensing with graphene edges

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Chapter 6

Conclusions and outlook

6.1 Conclusions

Graphene line and point defects are classified based on their molecular structures and were reviewed in the context of whether their local electronic properties and chemical reactivities would be useful for designing graphene-based sensors (Chapter 1). Graphene edges are another type of line defect in graphene and were integrated in a graphene field effect transistor biosensor to detect miRNA (Chapter 2). In this chapter, edge-dominated dielectrophoresis with graphene nanoribbon field-effect transistors were explored to overcome fundamental diffusion limits and to achieve exceptional sensitivity. Next, millimeter-thick epoxy encapsulation of monolayer graphene and mechanical fracture were used to construct a biosensor utilizing the distinct quantum capacitance of monolayer graphene edges (Chapter 3). To address the need for reusability in edge-based sensing, a ceramic-encapsulated, multilayer graphene platform was developed, which enabled the fabrication of a robust and reusable quantum-capacitive biosensor functionalized at the graphene edges (Chapter 4). Finally, a monolayer graphene–bridged polyaniline (Gr – PANI) electrochemical biosensor was developed, where the electrochemical signal of the targeted biomolecules was amplified by using the field effect in graphene (Chapter 5).

Chapter 2 describes the use of an edge along a graphene nanoribbon (GNR) to generate spatially localized electric-field gradients for dielectrophoretic enrichment. Owing to the atomically sharp geometry of the graphene edge, the applied AC bias produces a non-uniform electric field with pronounced field crowding at the edge. The combination of this sharp curvature and the 12nm HfO_2 back-gate dielectric enhances the local electric-field gradient ($\nabla|E|^2$), which is the driving term for dielectrophoresis. As a result, polarizable biomolecules experience a positive DEP force that directs them toward the high-field-gradient region at the graphene edge. Through electrochemical grafting of aryl diazonium intermediates followed by the nucleophilic substitution on an NHS-ester, the resulting NHS-terminated aryl layer was subsequently reacted with amine-modified DNA probes via amide coupling, enabling site-specific probe immobilization exclusively at the graphene edge. When coupled with AC field-induced dielectrophoretic (DEP) forces, these functionalized edges enabled an attomolar-level detection of a target miRNA by overcoming diffusion-limited kinetics.

Chapter 3 investigates a polymer-mediated fracture method to create graphene edges for sensing applications. In this approach, monolayer graphene is embedded within a millimeter-thick epoxy matrix and mechanically fractured in liquid nitrogen to selectively expose edge sites while fully encapsulating the basal plane, eliminating its contribution to measured capacitance. Electrical characterization of the exposed edges revealed a distinct Λ-shaped quantum capacitance profile, with a maximum at the Dirac point, in contrast to the conventional V-shaped behavior of the basal plane. This Λ-shaped profile indicates a high density of edge-localized electronic states near the Fermi level, which directly amplifies the electrical response to molecular binding and improves signal amplitude under small perturbations. To enable

molecular recognition, the edges were functionalized via electrochemically activated diazonium salts, attaching NHS ester groups for covalent immobilization of amino-modified DNA probes. Using miRNA-21 as a model analyte, the sensor achieved femtomolar sensitivity (1 fM), demonstrating that the combination of edge quantum capacitance and edge functionalization produces a sensitive biosensing platform.

Chapter 4 developed a multilayer graphene-edge biosensing platform by embedding reduced graphene oxide (rGO) flakes within a ceramic matrix, thereby creating a device with a high density of accessible graphene edges distributed throughout the structure. The rGO/SiO_2 powder nanocomposites were first prepared by mixing hexylamine-intercalated GO powder with TEOS, followed by hydrolysis, filtration, drying, and calcination under nitrogen flow, which partially reduced GO and converted the silica precursor to SiO_2. The resulting powder was densified into bulk nanocomposites using spark plasma sintering at 1350 °C under controlled pressure, ensuring that the graphene basal planes were fully encapsulated within the ceramic while leaving edges exposed. The accessible graphene edges were then selectively functionalized through reversible electrochemical grafting of aryl diazonium salts. Quantum capacitance measurements showed that the edge-enhanced graphene quantum capacitor (eGQC) sensors exhibited highly reproducible responses over 100 consecutive miRNA detection cycles, confirming the robustness and reversibility of the edge sensing application.

In Chapter 5, a graphene-bridged polyaniline (PANI) organic electrochemical transistor biosensor was designed. Vertically aligned PANI nanoarrays were electropolymerized on a monolayer graphene channel using a cyclic pulsed voltage protocol (−0.1 V for 1 s, 0.4 V for 2 s, and 0.8 V for 0.1 s per cycle) in an electrolyte containing 0.1 M aniline and 1 M $HClO_4$, achieving near-complete surface coverage after 200 cycles. DNA probes complementary to miRNA-21 were then covalently immobilized on the PANI surface via NHS ester-mediated amidation, and the device was passivated with 0.05% Tween-20 to block non-specific adsorption. The biosensor demonstrated an ultralow detection limit of 1 fM for miRNA. This high sensitivity arises from redox-driven modulation of the PANI upon miRNA hybridization, which alters local protonation and hole density, while the high carrier mobility of the graphene channel rapidly transduces these changes into measurable electrical signals.

6.2 Outlook

This thesis primarily investigates the use of graphene edges in the form of graphene nanoribbons, mechanically fractured monolayer graphene, and ceramic-embedded reduced graphene oxide which are further used to detect a model miRNA probe. Building on the progress demonstrated in this dissertation, several research directions could be proposed:

6.2.1 Characterization of functionalization density

A key challenge encountered in characterizing our graphene edge-based biosensing devices lies

in the precise control and reliable quantification of functionalization density at graphene edges. Specifically, when functionalizing graphene edges via diazonium chemistry, conventional characterization techniques face significant limitations in probing the molecular coverage specifically within the nanoscale edge region. For instance, conventional Raman spectroscopy, a widely used tool for chemical characterization, struggles to achieve this goal due to its insufficient spatial resolution, which fails to distinguish the nanoscale edge region. Additionally, the overwhelming Raman signal emitted from the graphene basal plane masks the weak signal from the edge-immobilized diazonium-derived groups, making it impossible to accurately quantify the functionalization density at the edges. This lack of precise quantification hinders our ability to optimize functionalization protocols. To address this critical characterization challenge, Tip-enhanced Raman spectroscopy (TERS) offers a promising solution. TERS combines scanning probe microscopy with plasmonically enhanced Raman scattering, enabling nanoscale chemical mapping with sub-nanometer spatial resolution. This unique spatial resolution allows for direct and quantification of diazonium-derived edge-bound groups, effectively eliminating interference from the graphene basal plane. By providing crucial feedback on the density, distribution, and uniformity of edge functionalization, TERS can facilitate the systematic optimization of our diazonium-based modification protocols, ensuring consistent and controllable edge functionalization across devices.

6.2.2 Density characterization of DNA probes at graphene edges

Quantifying the density of DNA probes is equally essential for ensuring sensor-to-sensor consistency, yet it remains experimentally difficult due to the small size of DNA strands and the fluorescence-quenching property of graphene. To overcome this, an alternative strategy can be implemented: gold nanoparticles (AuNPs) may be anchored to the graphene edge via amide bonding between amine-terminated DNA probes and NHS-ester groups pre-grafted on the edge. Subsequent scanning electron microscopy (SEM) imaging then allows direct visualization and statistical analysis of AuNPs distribution along the edge, thereby providing an indirect but reliable measure of DNA probe density.

6.2.3 Biosensing in complex environments

While graphene-edge biosensors exhibit extraordinary sensitivity in controlled buffer solutions, their practical application in real-world biological samples remain a significant challenge, such as serum and plasma. The primary obstacle stems from nonspecific adsorption of biomacromolecules (*e.g.,* proteins, lipids) present in complex matrices onto the highly reactive edge sites. Such fouling can induce false-positive signals, degrade long-term stability, and severely compromise the specificity and accuracy of detection. To translate laboratory-level performance into clinically viable devices, future efforts can focus on integrating effective sample-processing modules directly with graphene-edge sensing platforms. A promising

strategy involves the on-chip integration of size-exclusion or affinity-based filtration units that selectively retain large interfering species while permitting the passage of small-molecule analytes toward the sensing region. Such integrated microfluidic systems could be designed to perform continuous or batch-mode pretreatment, thereby minimizing nonspecific adsorption before the sample contacts the functionalized graphene edge.

6.2.4 Precise control of graphene edge type

Graphene edges are primarily composed of zigzag and armchair configurations, which exhibit distinct electronic and chemical properties due to their different energetic states. Zigzag edges host quasi-localized π states that produce a large density of electronic states (DOS) close to the Fermi level (E_F). In contrast, armchair edges preserve sublattice symmetry, resulting in delocalized electronic states that are distributed away from E_F, thus yielding a lower DOS at the Fermi level compared to zigzag edges. Since the change in stored charge under a small perturbation obeys $\Delta Q = C_Q \Delta V$, where ΔV represents the potential shift induced by charged biomolecular adsorbates, a larger C_Q directly amplifies the electrical response to molecular binding, improving signal amplitude. Moreover, zigzag edges are chemically more reactive than armchair edges. This higher reactivity facilitates denser and more robust covalent functionalization, which increases the density of probe molecules (more binding sites per unit edge length). Therefore, the development of edges with well-defined zigzag structure would represent an interesting direction to explore.

6.2.5 Potential applications of POCT in clinical diagnostics and portable devices

Beyond fundamental performance optimization, graphene edge-based quantum-capacitive biosensors hold considerable promise for translational applications in point-of-care testing (POCT), clinical diagnostics, and portable analytical devices. Their exceptionally high sensitivity, label-free operation, and compatibility with miniaturized device architectures make them particularly attractive for rapid biomarker detection in resource-limited environments. Compared with conventional laboratory-based assays, edge-enabled graphene sensors offer the potential for shorter turnaround time, reduced reagent consumption, and lower instrumentation complexity, which are all key requirements for decentralized testing platforms.

From a commercialization perspective, the main advantages of graphene edge-based sensing include scalable fabrication, low power consumption, and the possibility of multiplexed detection through device-array integration. However, several challenges remain before clinical translation can be achieved, including robust control of edge functionalization, reproducible large-area manufacturing, operation in complex biological matrices, long-term stability, and standardization of device calibration. Addressing these issues will be essential for advancing graphene-edge based biosensors from proof-of-concept demonstrations to clinically deployable and commercially viable diagnostic technologies.