**Supplementary Materials**

**Graphene Quantum Dots Electrochemistry and Development of Ultrasensitive Enzymatic Glucose Sensor**

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Materials and methods

*Synthesis* Graphene oxide (GO) was prepared by a modified Hummer’s method, followed by chemical reduction using hydrazine monohydrate to produce reduced GO (rGO).[41] We synthesized graphene quantum dots (GQDs) using solvothermal route[44] and hydrothermal method [17-19], wherein both GO and rGO as precursors were used (see Ref. [20] for details). Briefly, for solvothermal preparation, 0.5-g GO and 50-mL of N-N dimethylformamide (DMF) produced 10 mg/mL concentrated GO/DMF suspension, ultrasonicated for 1 h, transferred into a 60-mL Teflon-lined stainless steel autoclave, and heated in a muffle furnace at 140 and 200 °C, for 8 h. The final GQD/DMF product was obtained through vacuum filtration using a 0.2-μm micropore membrane. The GQD/DMF suspension was roto-evaporated to remove DMF and to obtain GQDs, which were then re-dissolved in pure water and phosphate-buffered saline (PBS) to produce different suspensions. The GO/DMF of 0.5, 1, 5, 10, and 20 mg/mL were strictly controlled with 40%, 60%, and 80% ratios and reaction times of 8h and 12h. To select the optimal preparation conditions, the fluorescence quantum yield of different GOD samples was measured using fluorescence spectroscopy (excitation wavelength of 420 nm) with DMF as reference. For hydrothermal method, GO nanosheet was partially deoxidized in a tube furnace at < 300 °C for 2h in Ar atmosphere. The thermally reduced GO nanosheet dispersions of 1.0 mg/mL in deionized (DI) water was prepared by stirring 8h and mild ultrasonication for approximately 40 min. These dispersions were purified with microporous (0.2 μm) polytetrafluoroethylene (PTFE) membrane and re-dispersed in DI water. Then the suspensions were heated at 200 °C, 170 °C and 140 °C for 8-10h in a Teflon-lined stainless steel autoclave. The resulting black suspensions were filtered with PTFE membrane and a dark brown filtered solution was obtained. To remove larger graphene nanoparticles, the colloidal solution was dialyzed retaining molecular weight, 3500 Da overnight. The GQDs obtained from these two precursors showed stability over six months or longer. These dispersions were drop cast followed by air dry on commercial substrates, holey grids and on glassy carbon (GC) electrodes as needed for various structural characterization and electroanalytical studies.

*Characterization* Samples were characterized at nanoscale to obtain size distribution, crystallinity, optical and lattice vibration properties. Samples for high-resolution transmission electron microscopy (HR-TEM) were prepared by placing two drops of GQDs on commercial Cu grids coated with lacey carbon (Ted Pella Inc., Redding, CA, USA) and allowing them to air dry. They were taken using a JEOL instrument (Model 1400 Plus, OR, Peabody, MA, USA) operating in Cryo-EM mode at 200 kV and 1 nA with a Be specimen holder and a Gresham SiLi detector with Moxtek AP3.3 window. The measurements were also performed in the STEM mode using a nanoprobe with probe size of ~1 nm. STEM images were collected with a Fishione HAADF (High-Angle Annular Dark-Field) detector and EDX (Energy Dispersive Spectroscopy) signals were measured using an EDX detector yielding C/O ratio of 8:1 for both GQDs prepared using GO and rGO. Interestingly, GO is reduced while undergoing hydrothermal treatment. The optical (UV-Vis absorption and fluorescence) spectroscopy measurements were taken using a BioTek spectrometer (Model Synergy H1 Multi-mode Reader, Winooski, VT, USA) equipped with a xenon lamp as broadband excitation source. For fluorescence measurements, the excitation wavelength *λ*ex = 370 nm and spectral window of 350–550 nm was used with wavelength interval 0.5 nm and for UV-Vis the absorption spectroscopy is measured between 210 and 550 nm in interval of 1 nm. All the measurements were carried out at room temperature (~298 K). X-ray diffraction (XRD) patterns were obtained with Siemens Model D5000 instrument (Thermo Scientific, MA) in Bragg-Brentano θ-2θ geometry ranging 2θ from 8° to 30° using Cu Kα X-ray source (λ=1.5405 Å) operating at voltage of 45 kV and current 40 mA. Samples were run at a scan rate of 0.04 °/s or to improve scattering signal counts, we measured at 0.02°/s scan rate. Raman spectroscopy was carried out to determine the lattice vibration structure at various points on GQDs and other samples. The Raman spectra were recorded using a micro-Raman spectrometer (Model InVia Renishaw plc, Gloucestershire, UK) equipped with laser excitation wavelength 633 nm (*E*L = 1.92 eV) and ~1–2 mW power incident at the sample, with edge filters cutting at ~100 cm−1 and an objective lens of 50× providing spot size ~2 μm. The scattered light from the sample is collected in backscattering geometry, transmitted by a beam splitter, and detected by a CCD camera. Extreme care was taken to avoid sample damage caused by laser-induced thermal degradation and therefore 5% or 10% light intensity was used to obtain spectra with acquisition time per pixel ranging from 60 s to a few minutes, though it was increased to 300 s to optimize the signal-to-noise ratio. Raman shift ranged from 1100 to 3200 cm−1 with a spectral resolution of 1 cm−1.

*Preparation of GQD-modified and GOx-immobilized electrodes* Glucose oxidase, GOx (from Aspergillus niger, E.C.1.13.4) was obtained from Sigma-Aldrich (St. Louis, MO, USA) and the stock solution was prepared in 50 mM phosphate buffer solution (PBS) pH 7.8 and stored at 4 °C. D(+)-glucose, ferrocene methanol (FcMeOH) and glassy carbon (GC) 4 mm diameter rods were purchased from Merck Chemicals (Kenilworth, NJ, USA) and Alibaba Co. (Hangzhou, China), respectively. All other chemicals were of analytical grade and used without further purification. Due to the unique properties of GCs—good electrical conductivity, renewable/reusable electrode surface, mesoscale porosity, and cost effectiveness—they were used to immobilize graphene nanosheets, graphene quantum dots, enzymatic proteins, and combinations thereof for electrochemical and biosensing investigations. The as-received GCs were shined with alumina paste and a polishing cloth, and subsequently washed and rinsed several times with DI water. The GQD solution (50 μL), GO and rGO (both 70 μL) were placed thrice using a Hamilton syringe on GC substratesand dried overnight at room temperature to obtain homogeneous GQD|GC, GO|GC and rGO|GC samples. To activate these electrodes, pre-treatment was performed electrostatically at +1.5 V for 100 s. Finally, 20–25 μL of GOx, Mb (Myoglobin), Cytc (Cytochrome c), and HRP (all 2.0 mg/mL concentration in DI water) were immobilized via physical adsorption using a Hamilton syringe to prepare GOx-GQD|GC, GOx-GO|GC, GOx-rGO|GC, Mb-GQD|GC, Cytc-GQD|GC, HRP-GQD|GC electrodes. For slow evaporation of water and also for uniform film formation, the electrodes were covered with a petri dish. All of the modified electrodes were stored at 4 °C in refrigerator when not in use.

*Microstructure and optical properties*

The crystalline structure of GQDs is investigated by X-ray diffraction (XRD) and micro-Raman spectroscopy (RS) shown in Fig. S1. The GQDs contain similar oxygenated functional groups as their precursors, including carbonyl (-C=O), carboxyl (-COOH), epoxy (-O-), and hydroxyl (-OH), distributed at the multilayer terrace or edge planes and preferential reduction produces reduced GQDs. The energy-dispersive X-ray spectroscopy (EDX) spectra (not shown) yielded C to O ratios ranging between 55.34 and 69.27 at.% as anticipated. The interlayer distance of GQDs calculated from the XRD peaks position at 2θ=12.9o and 14.3o assigned to (002) atomic plane correspond to d1= 0.685 nm and d2= 0.619 nm, respectively.

UV-Vis absorption and photoluminescence spectroscopy (see Fig. S1) revealed the characteristic bands associated with as-prepared GQDs (Figure S1). Broad weaker absorption bands at ~305 nm and at ~340 nm and photoluminescence peaks at 430 (2.87 eV) and 460 nm (2.68 eV), excited at 370 nm (3.34 eV), are characteristic of apparently blue-violet GQDs according to various reports.[26,43] The absorption band at ~278 nm corresponds to π (bonding)−π\* (antibonding) transition (characteristic of natural π-conjugated graphene sheets) of aromatic sp2 C domains. Like most of the work reported, as-synthesized GQDs possess excitation wavelength-dependent PL (PLE) showed in Figure 1f, where the spectral maxima shift with excitation energy. In addition, the peaks at 305 nm and 340 nm do not shift with excitation energy andare independent of reaction duration, thus the size of GQDs does not matter. Therefore, the variable PL maxima and and the invariable peak at ~340nm suggest multiple emissive mechanisms responsible for the overall emission from GQDs. It is proposed that the blue shift of PL maxima is due to quantum confinement of excitons, according to which the smaller the GQDs size, the wider the bandgap and the higher the emission energy [45,46]. Moreover, the oxygenated functional groups combined with atomic scale defects produce irregularly hybridized π states, which causes so-called energy states induced by structural deformation (*ESiD*). These *ESiD* possess energy levels in-between the HOMO/LUMO (Highly Occupied Molecular Orbitals)/ Lowest Occupied Molecular Orbits) gap that serve as intermediate or mid-gap states between bonding and anti-bonding states. All of the microscopic structural characterization results indicate that high crystalline quality GQDs are synthesized successfully.

*Electrochemistry* A custom designed three-electrode electrochemical cell was used with a bi-potentiostat electrochemical workstation (CH Inc. Model 920D, Austin, TX, USA) for electrochemical measurements in cyclic voltammetry (CV), differential pulse voltammetry (DPV), and *ac* electrochemical impedance spectroscopy (ac EIS) modes, where a saturated Ag/AgCl (3M KCl) electrode and Pt wire (3 mm diameter) were used as reference andcounter electrodes, respectively. These techniques were used to investigate electrode kinetics and in turn assess the working electrodes’ performance. The GQDs and other graphene-based samples were used as working electrodes characterized in 0.1M PBS electrolyte within the potential range −0.9 and +0.9 V at various scan rates *v* = 5, 10, 20, 50, 100, 200, 300, 400, 500 mV/s in CV mode. The *ac* EIS measurements were conducted in the frequency range 10 mHz–98 kHz at +0.3 V superimposed with alternating current voltage amplitude 10 mV. For GO*x* sensing, the CV measurements were carried out from −0.8 to 0.2 V at a 20 mV/s scan rate with successive addition of glucose concentration (0.5, 1, 1.5, 2.0, 2.5 and 3 mM) for GO*x*–GQD|GC. The differential pulse voltammetry (DPV) was also used for enhanced sensitivity between potential range −0.8 and +0.8 V at *V*amp = 25 mV. Amperometry (*i*–*t*) monitored the current with discrete additions of 10 and 100 μM glucose at a constant potential of −0.4 V in 0.1 M PBS, pH 7.4 at intervals of 50 s until 1200 s on various electroanalytical platforms [GO*x*-(GO, rGO, GQD)|GC]. Scanning electrochemical microscopy (SECM) was employed to gain further insights into the electrode/electrolyte physicochemical interfacial processes and quantify the associated parameters [44]. Briefly, SECM measurements are carried out in cyclic voltammetry, probe approach in negative feedback modes using the same bi-potentiostat mentioned above. This technique uses a Pt microelectrode (~5 μm) tip as working electrode 1 with 5 mM ferrocene methanol (FcMeOH) redox probe in support electrolyte 0.1 M PBS. FcMeOH has a standard potential *E*0 = +0.21 V versus Ag/AgCl. The Pt tip electrode was held at a potential of *V*t = +0.5 V to ensure complete diffusion-limited oxidation of Fe(II) species originally present in the electrolyte solution to Fe(III) with substrates as working electrode 2. For SECM imaging method, the electrodes (tip and substrate) were biased at *V*t = +0.5 V and *V*S = −0.4 V, respectively. The tip was rastered over the working electrode 2 (GQD, GO*x*−GQD, HRP−GQD, Mb−GQD, Cyt *c*−GQD, Cyt *c*−GO and GO*x*−rGO) surface area (500 μm × 500 μm) kept at a constant tip–substrate separation ≤8–10 μm to generate a feedback image with an approximate resolution of tip radius with a sub-nanoampere level of current sensitivity. These studies were performed several times on multiple electrodes and the results were reproducible within <98% for all of the electrodes.

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**Figure S1**. (c) X-ray diffractogram and (d) Raman spectra of GQDs prepared from GO at various synthesis temperatures, (e) UV-Vis absorption (190−550 nm) and room temperature photoluminescence spectrum of GQD showing optical emission in blue-violet region (inset shows GQD vials without (clear) and with (blue violet) UV lamp); and (f) photoluminescence excitation (PLE) spectra of GQDs.