DNA-Directed Self-Assembly of Graphene Oxide with Applications to Ultrasensitive Oligonucleotide Assay

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ABSTRACT Controlled graphene or its derivatives' assembly is of growing interest in many areas. However, achieving control over their assembly into precise and predictable architectures has been challenging and is still a bottleneck to their utilization. Herein, we report for the first time the use of DNA hybridization for the controllable assembly of a graphene nanosheet. Moreover, with the help of dynamic light scattering technique, we extended the above studies by exploiting the DNA–graphene dispersed sheets as highly ultrasensitive detection of oligonuleotides for the fabrication of a novel biosensing strategy, which shows high sensitivity and excellent selectivity. This work will show a new general route to graphene-based lamellar composite materials and would bring about advances in the research of graphene-based biofunctional materials for specific applications in biodiagnostics, nanoelectronics, and bionanotechnology.

KEYWORDS: graphene oxide · DNA · self-assembly · oligonucleotide assay

Controlled molecular assemblies provide ideal "bottom-up" approaches to new functional materials and devices with desirable organization.[1−3] The use of biological recognition of peptide[4] and nucleic acid[5,6] represents a particular useful option for precise control of the assembly processes.[4−10] This biomimetic strategy has been exploited for the development of various exquisite structures from nanoscale components such as gold nanocrystals,[8] quantum dots,[9] and carbon nanotubes.[10] Graphene, a kind of most exciting monolayer material of sp^2-bonded carbon, has attracted increasing attention in fundamental and application-oriented research because of its excellent electrical, mechanical, and chemical performances.[11−18] Currently, many attempts have been made to design and construct hierarchical graphene-based materials, for example, coassembly of graphene and titania nanosheets or proteins *via* electrostatic interactions.[16−18] These reported routes lack information-containing molecules to efficiently guide the assembly process. Considering the layered assembled graphene-based structure, the parallel orientation is favorable for multivalent biomolecular recognition with biological synergy of specificity and affinity, which can facilitate the controllable assembly of graphene into precise and predictable architectures. However, to the best of our knowledge, the biometric-mediated assembly of graphene has not been reported up to now.

DNA is one of the most promising chemical moieties to direct the self-assembly process.[5,6] Due to its double helix structure and the base pairing, DNA-mediated assembled structures can be designed and controlled as a desirable and reversible route. Recent studies[19−22] have demonstrated that single-stranded DNA (ssDNA) can be used for binding graphene and its derivatives *via* strong interactions including van der Waals forces, $\pi-\pi$ stacking, and/or hydrogen bond. In this article, we report for the first time using DNA hybridization for controllable assembly of the nanosheets of graphene oxide (GO), as shown in Scheme 1. Two single-stranded sequences DNA_1 and DNA_2 are separately added to GO solutions to form DNA_1–GO and DNA_2–GO complexes, respectively. DNA_1 has a target-specific sequence complementary to target DNA (T-DNA) and a $d(GT)_{15}$ tail at the 5′ terminus to facilitate the assembly of DNA_1 on GO nanosheets. DNA_2 also has a complementary sequence to target T-DNA with a $d(GT)_{15}$ tail at the 3′ terminus. In the mixture of the two DNA_1–GO and DNA_2–GO complexes, T-DNA will hybridize with both DNA_1 and DNA_2, which results in an assembly of the GO nanosheets. Such assembly process is expected to produce the layered structure with tunable interlayer spacing. In particular, it was observed that the size of the GO assembly was concentration-dependent and base-mismatch-sensitive to target DNA, as revealed by dynamic light scattering (DLS) technique.[23,24] This allows us to develop a novel biosensing platform for highly sensitive and selective detection of target DNA.

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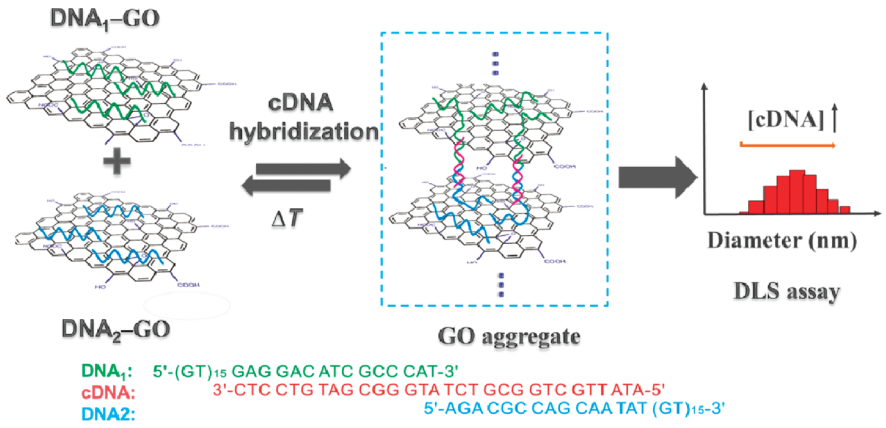

Scheme 1. Schematic illustration of procedures for DNA-directed self-assembly of graphene oxide and a homogeneous detection of DNA by dynamic light scattering (DLS) technique. Note that the as-made GO aggregate could also be disassembled by thermal treating.

RESULTS AND DISCUSSION

In the biosensing system, GO and DNA–GO complexes were prepared with procedures[25] found in Supporting Information. After centrifuging and purification steps, we obtained a well-dispersed and high-quality GO sample. The typical morphology of GO is shown in atomic force microscopy (AFM) and transmission electron microscopy (TEM) images (see Figures S1 and S2), indicating that the thickness of the graphene layer is around 1.5 nm, corresponding to the monolayer GO sheet. Moreover, characterization of a large-area GO sample from Raman spectra and Fourier transform infrared spectroscopy (FTIR) exhibits the high content of oxygen-containing groups in the GO layer (Figures S3 and S4). Next, DNA–GO complexes were prepared by incubation of the as-prepared GO with ssDNA (DNA_1 or DNA_2) and then ultracentrifugation to remove free DNA. As shown in previous reports,[17] in this colloidal system of DNA–GO, the existence of DNA can improve the dispersion behavior and stability of GO in a wider range of pH, ionic strength, and temperature. As the DLS measurements show in Figure S5, the resulting DNA–GO complexes exhibit good stability in the Tris-boric acid buffer solution even at a high salt concentration.

Upon mixing the DNA–GO complexes with 10 pM T-DNA, large aggregates were immediately obtained due to the hybridization of DNA_1 and DNA_2 with T-DNA, as observed in AFM and TEM investigations (Figure 1). The height of section extracted from the typical AFM image along the layer edge indicates that the DNA-mediated GO aggregate was 3–20 nm in apparent thickness (Figure 1A), whereas the DNA–GO complex presented a layer thickness of ~1.8 nm (Figure 1B). It is obvious that such aggregate height data were consistent with the thickness of a multilayers of DNA functional GO nanosheets. Moreover, a typical TEM image of this GO aggregation also reveals its well-packed layered structure with a clear single-layer resolution (Figure 1C). The above results suggested that the significant variation of GO thickness was closely correlated to the DNA hybridization.

By evaporation-induced assembly or vacuum filtration, a thin self-supporting film of the resulting GO aggregate was obtained. X-ray diffraction (XRD) was employed to characterize its crystal structure, as shown in Figure 1D. The intense crystalline peak of GO film occurs at 10.7°. This is the characteristic peak of hexagonal graphite with a d spacing of 0.830 nm. Instead, the XRD profile of as-obtained GO aggregate film shows a strong single reflection at $2\theta = 4.35°$, corresponding to an expanded interlayer spacing (d_{001}) of 2.03 nm. This significant increase in d spacing is due to the intercalation of DNA molecules between the GO layers. Therefore, all of the results of microscopy and XRD confirmed that the DNA hybridization process can induce the DNA–GO assembly and result in a layered bionanostructure.

To investigate the formation of DNA–GO complexes and its aggregate resulting from DNA hybridization, fluorescence and fluorescence anisotropy measurements were used. As shown in Figure 2a, when adding a certain amount of GO, the fluorescence of Cy3-labeled DNA (Cy3–DNA_1) exhibited substantial fluorescence quenching (>90%), suggesting a strong and rapid adsorption of DNA onto the graphene surface. The fluorescence quenching is contributed to the electron or energy transfer between the dye and GO surface. Additionally, fluorescence anisotropy (FA) is commonly used to probe molecular interactions, which can differentiate the molecular weights of the fluorophore. So, the measured FA values of Cy3–DNA_1 and Cy3–DNA_1–GO complex, about 0.065 and 0.198, respectively, illustrated direct evidence of the adsorption of DNA on GO. After incubating target DNA with the Cy3–DNA_1–GO and DNA_2–GO mixture, it was also observed that the FA values increased from 0.208 to 0.489 following increased concentration of target DNA

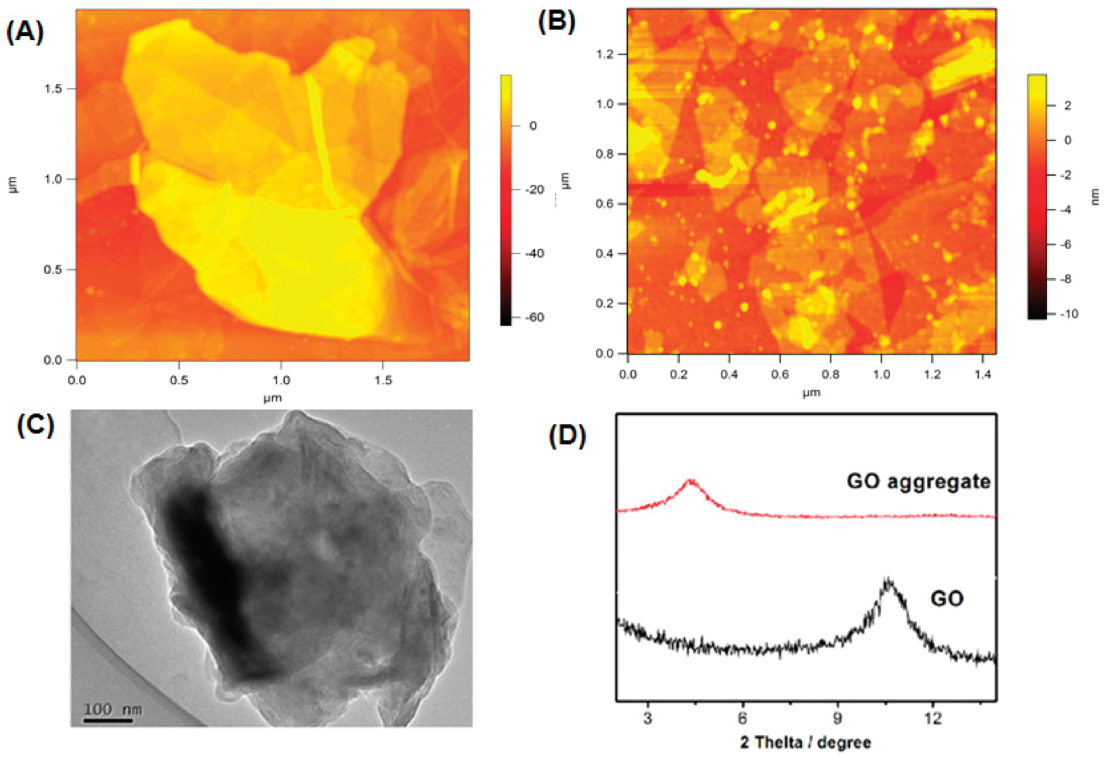

Figure 1. Tapping mode AFM images of DNA−GO complex (A) and DNA−GO aggregate formed from DNA_1−GO and DNA_2−GO mixing complexes with 10 pM T-DNA (B). (C) High-resolution TEM image of DNA−GO aggregate showing its typical multilayer-stacked morphology. (D) XRD patterns of GO and DNA−GO aggregate film exhibiting their crystal structure.

(T-22) (Figure 2b), implying its regular growth of the GO aggregate. Altogether, these results demonstrated that DNA could bind on GO with a strong interaction affinity and further clarified that the GO assembly process is mediated by DNA hybridization.

DLS technique was also used to understand the DNA-directed GO assembly process. As known, DLS is photon correlation spectroscopy or quasi-elastic light scattering, which is based on the Brownian motion of spherical particles, which causes a Doppler shift of incident laser light. The diffusion constant of particles is measured, and the size of the particles is calculated according to the Stokes−Einstein relation. This technique is used routinely to analyze the size and size distribution of polymers, proteins, colloids, and nanoparticles.[23,24] Note that here the DLS data used a sphere model to simulate GO nanosheet assemblies. So the obtained hydrodynamic diameter values could not accurately reflect the absolute *Z*-average sizes of the nanosheet assemblies. Nevertheless, such apparent values might exhibit close correlation to the relative physical sizes of the GO assemblies.[6,23,24] As shown in Figure 3a, after incubation with 10 pM target DNA (T-22) for 2 h, the *Z*-average diameter for DNA−GO increased from 212 to 433 nm, implying the formation of aggregate. Moreover, subsequent heating at 65 °C resulted in dissociation of the duplex and a decrease of aggregate scale (Figure 3a), indicating that such DNA hybridization-mediated GO assembly is a thermally controllable and reversible process. In addition, we observed that different lengths of target DNA had very obvious effects on the sizes of GO aggregates. With lengths too short (10−14mer), no obvious aggregation was observed due to its low T_m, whereas for the longer target DNA (>22mer), the stable hybridization could induce GO assemblies of very large size (Figure 3b). Surprisingly, DLS analysis of the aggregate sizes at different temperatures revealed that the melting temperature of GO−DNA is substantially higher than the melting point of DNA_1 (or DNA_2) with T-DNA (Figure S6 in Supporting Information). This is analogous to the hybrid of DNA−Au nanoparticles with an enhanced stability of DNA duplex, as reported by Mirkin.[26,27] We supposed that the layer-by-layer assembly structure may benefit the cooperative binding of the oligonucleotides between the graphene sheets. Such multivalent interaction will significantly improve the thermal stability of GO aggregates.

More importantly, we observed that the resulting GO aggregate sizes were closely correlated to the target DNA concentration (Figure 4).[23,24] As seen in Figure 4a and Figure S7, with increased concentration of target DNA, the average GO assembly hydrodynamic diameter increased accordingly with a wide dynamic detection range from 1 pM to 10 nM and a readily achieved detection limit of 1 pM. The relative standard deviation was ~4.2% across four repetitive

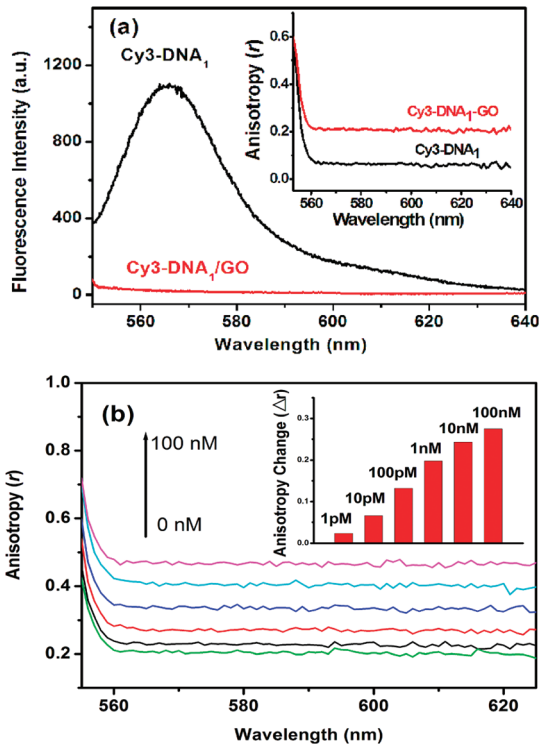

Figure 2. (a) Fluorescence intensity and anisotropy (inset) of Cy3−DNA_1 and Cy3−DNA_1−GO conjugate in 50 mM Tris-boric acid buffer solution (pH 8.0). (b) Fluorescence anisotropy of the Cy3−DNA_1−GO and DNA_2−GO mixture solution in the presence of target DNA at different concentrations. Inset is the average fluorescence anisotropy change plotted against the target DNA concentration. Excitation wavelength: 530 nm.

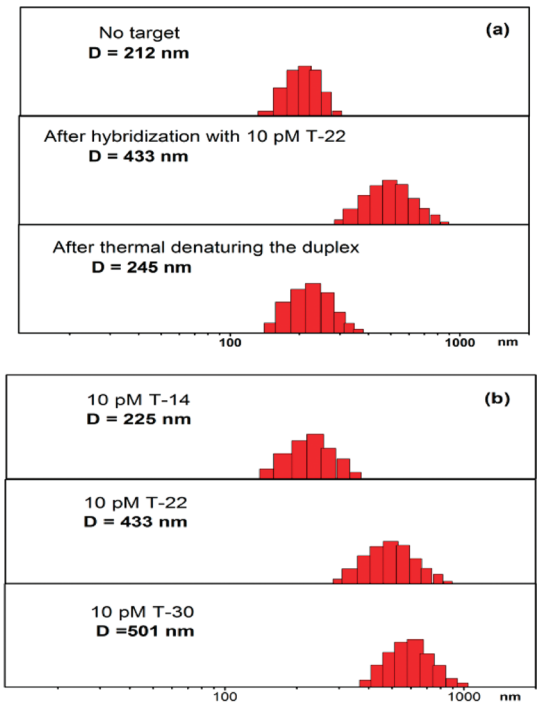

Figure 3. Size and size distribution (in diameter, nm) of DNA−GO assay solutions before and after hybridization by the target DNA (T-22), and then experienced thermal denature (65 °C, 30 min) (a), and in the presence of different lengths of target DNA (b).

assays of 10 pM, indicating that the DLS assay exhibited excellent reproducibility. Moreover, two types of mismatched DNA were chosen to examine the specificity of this assay. As shown in Figure 4b and Figure S8, the mismatched DNA either with 22 or 30 bases did not give aggregates under the same condition. The results demonstrate that the single-base-mismatched DNA strands could be directly discriminated from the perfectly complementary targets, indicators of high specificity of the proposed DLS assay. This high specificity seems to be attributed to the synergic and multivalent effects of the DNA−GO complexes in hybridization with target DNA, in which a single-base mismatch led to synchronous decrease of affinity at all binding sites between DNA_1 and T-DNA. Therefore, it is possible to use this DNA-mediated GO assembly as a robust biosensing platform for highly sensitive and selective detection of target DNA.

CONCLUSION

In conclusion, we have developed a novel strategy for controllable self-assembly of GO using DNA hybridization, which was confirmed thoroughly by AFM, TEM, XRD, and spectra measurements. Moreover, coupled with DLS technique, such GO assembly can be used as a one-step homogeneous biosensing platform

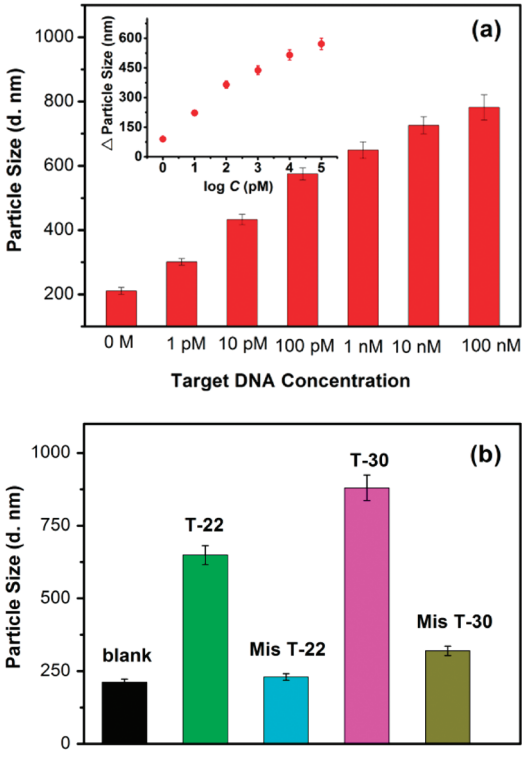

Figure 4. Average diameters of GO assembly as determined from DLS measurement in the presence of perfectly matched target DNA (T-22) at different concentrations (0 nM, 100 nM, 10 nM, 1 nM, 100 pM, 10 pM, 1 pM) (a) and single-base-pair-mismatched DNAs at a concentration of 1 nM (b). Inset is the average diameters plotted *versus* the target DNA concentration.

for DNA detection with high sensitivity and superb selectivity. The use of biomolecular recognition for GO assembly may provide an easy and facile route to manipulate or even control the assembly of graphene and its derivatives. This holds great promise for selective detection of the biomolecules of medical relevance. It is believed that this work will significantly advance the research of graphene-based biofunctional materials for specific applications in biodiagnostics, nanoelectronics, and bionanotechnology.

MATERIALS AND METHODS

Materials. Graphite powder (99.99995%, 325 mesh) was purchased from Alfa Aesar. Other chemicals, $KMnO_4$, NaOH, KCl, and NaCl, were from Beijing Chemical Company. Ultrapure water was obtained through a Nanopure Infinity ultrapure water system (Barnstead/Thermolyne Corp., Dubuque, IA) and had an electric resistance of >18.3 MΩ. The oligonucleotides used in this work were obtained from Takara Biotechnology Co. Ltd. (Dalian, China). The sequences of the synthesized oligonucleotides are given in Table S1 in Supporting Information.

Synthesis of Graphene Oxide (GO). The preoxidized graphite was reoxidized by Hummers' method. Pretreated graphite powder was put into 0 °C concentrated H_2SO_4 (120 mL). Then, $KMnO_4$ (15 g) was added gradually under stirring, and the temperature of the mixture was kept below 20 °C by an ice bath. Successively, the mixture was stirred at 35 °C for 4 h and then diluted with deionized water (250 mL) by keeping the temperature under 50 °C. Water (700 mL) was then injected into the mixture followed by adding H_2O_2 (30 wt %) (20 mL) drop by drop. The mixture was filtered and washed with an aqueous HCl solution (v/v 1:10) (1 L) to remove metal ions followed by deionized water to remove the acid. The resulting solid was dried in air and diluted to make a GO dispersion (0.5 wt %). Finally, it was purified by dialysis for 1 week to remove the remaining metal species. Exfoliation was carried out by sonicating the GO dispersion (0.1 mg/mL) under ambient conditions for 1 h.

Synthesis of DNA_1–GO and DNA_2–GO Conjugates. In a typical procedure, GO aqueous solution (1 mL, 0.1 mg mL^{-1}) and DNA_1 or DNA_2 (5 OD) was mixed by sonicating for 2 h and then incubated at room temperature for 24 h, and the concentration of NaCl was slowly increased to 0.01 M during the incubation. To remove free unbound DNA_1 or DNA_2, the solution was centrifuged at 16 000*g*, the supernatant was discarded, and the precipitated DNA_1–GO and DNA_2–GO conjugates were then redispersed in 0.5× TBE buffer (Tris, 44.5 mM; boric acid, 44.5 mM; EDTA, 1 mM; pH 8.0). This precipitation–redispersion process could be repeated several times to guarantee a complete removal of free DNA.

Hybridization of DNA_1–GO and DNA_2–GO with Target DNA. DNA_1–GO and DNA_2–GO conjugates (100 μL) were mixed in a 1:1 ratio, and target DNA (10 μL) and one-base-pair-mismatched DNA solution with different concentrations were then added respectively. The mixture remained in TBE buffer for 12 h at room temperature. Then, a drop of solution (20 μL) was diluted with DI water (100 μL), and with DLS measurement was conducted.

Characterization. The transmission electron microscope (TEM) images were obtained with a Hitachi model H-800 transmission electron microscope opened at an accelerating voltage of 100 kV. The samples mixed with 5 μL of ethanol were dropped on a Cu grid and left to dry in air condition for 10 h. XRD patterns of graphene oxide and DNA–GO were obtained *via* a D8 Advance (Bruker) X-ray diffractometer with Cu Kα radiation ($\lambda = 1.5418$ Å). Scanning electron microscopy (SEM) images were obtained using a LEO 1530 field emission SEM system (Germany). Raman spectra were obtained using a confocal microprobe Raman system (Renishaw, RM2000). Fluorescence measurements were done with a F-7000 Hitachi spectrometer. For AFM characterization, typically, a sample for AFM imaging was prepared by first treating a freshly cleaved mica surface with 1 M $MgCl_2$ for 1 min, followed by addition of 10 μL of a sample solution onto the mica surface. The mica substrate was tilted to allow the droplet to spread on the surface. After adsorption for 1 min, the mica surface was washed twice with doubly distilled water and dried with compressed air. The sample was then scanned in tapping mode with a Nanoscope III Digital Instrument atomic force microscope (AFM). Dynamic light scattering (DLS) was measured by Malvern Instruments, equipped with the Dispersion Technology Software 5.03. The DLS instrument was operated under the following conditions: temperature 20 °C, detector angle 90°.

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Supporting Information Available: Synthesized oligonucleotide sequences, AFM image, TEM image, FTIR and Raman spectra of as-prepared graphene oxide, stability study of DNA–GO complexes at different salt concentrations, DLS analysis data of DNA–GO aggregate after thermal denaturing at different temperature or with different target DNA, and so on. This material is available free of charge *via* the Internet at http://pubs.acs.org.

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