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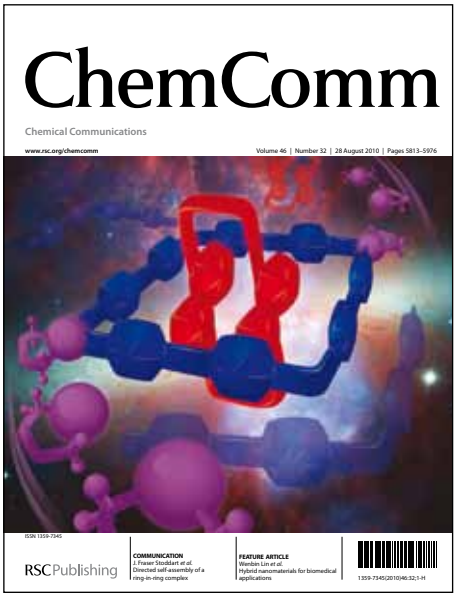

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Graphene Oxide/DNA Duplex−Based Logic Gate and Sensor Mediated by RecA-ssDNA Nucleoprotein Filaments

Longhua Tang, [1,2] Dongyang Li,[3] Jinghong Li [1]*

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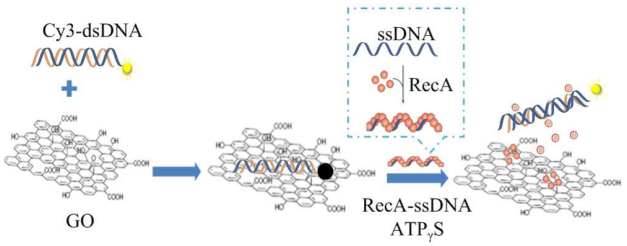
A conceptually new class of DNA logic gate and biosensor using the complex of graphene oxide/DNA duplex as a scaffold was reported, achieving by means of formation of the triple-stranded DNA structure mediated by Recombinational protein A (RecA)−ssDNA nucleoprotein filaments with graphene oxide.

Molecular logic gates and molecular computational systems are thought to be the future of computer technologies, which have used a variety of recognition mechanisms and may also be applied to disease diagnostics, bioengineering and nanomedicine.[1-3] Because of its molecular recognition ability, well-regulated structures and the ability to store genetic information, nucleic acids (DNA or RNA) logic gates have been proven to be highly active components for the design and construction of biocomputational devices with Boolean functions.[4-8] Up to date, diverse DNA logic gates have been proposed, most of which are based on base pairing or functional DNA recognition activity.[5-8] With significantly advance of the nano- and biotechnology, the integration of biomolecular logic gates with nanomaterials for the molecular computation has attracted considerable attention.[9] Although encouraging progress has been made in this field, to make simple, sensitive, and selective DNA/nanostructures hybrid-based logic gates is still a great challenge.

Among the carbon-based nanomaterials such as fullerenes, nanodiamonds, carbon nanotubes, and graphene, graphene attracts the most fundamental and practical interest in recent years.[10-16] The exceptional structural, electronic and mechanical properties make graphene as a promising material for future optoelectronics and nanoelectronic applications.[10] In particular, graphene or its water-soluble derivative have been recognized as an excellent platform for DNA-based bioassay and molecular logic gates because of the existence of non-covalent π-stacking interactions between the hexagonal cells of graphene and the ring structure of nucleobases.[12, 13] Previous work has shown that the conformational change of ssDNA binding on graphene oxide (GO) surface during target recognition will occur, which is the basis of analytical application.[12-16] For instance, complexes of ssDNA/GO have been successfully employed for biomedical and bioassay applications with targeting of nucleic acids, proteins, metal ions, small molecules, etc..[15] Most recently, we demonstrated that GO could also adsorb DNA duplex by controlling the concentrations of GO and DNA, which is possibly facilitated by partial deformation of the double helix on GO.[13] Additionally, DNA duplex on graphene shows specific biological effects on enzymatic degradation. Despite the progress,[15] to the best of our knowledge, there is still little work to be explored on the utility of GO/DNA duplex, such as their sensing capabilities or logic gate behaviors.[16-18]

Considering the biological significance of nucleic acids, the DNA duplex-GO complexes would provide a versatile and facile platform to investigate the interaction between DNA and other biomolecules, such as DNA, protein and enzyme. [15] In this work, therefore, a novel DNA biosensing-based logic gate strategy using GO/dsDNA complex as a scaffold was designed. As schematically represented in Scheme 1, this biosensing and logic gate platform was constructed via a triple-stranded DNA formation reaction between dsDNA absorbed on GO surface and RecA protein-ssDNA filament in homogeneous solution. RecA from *Escherichia coli*, the key protein in homologous recombination, has a significant role in genetic recombination, DNA repair, UV-induced mutagenesis.[19-21] For a course of recombinational DNA repair, RecA proteins first assemble on ssDNA and then search for homologous duplex DNA; subsequently the homologous strand exchanging occurs along of ATP hydrolysis.[22-26] In our sensing strategy, fluorophore-labelling DNA duplex (Cy3-dsDNA) first assembles on GO surface, which results in an efficient fluorescence quenching; while adding RecA-ssDNA nucleoprotein filament into the above solution and incubation in the presence of adenosine-5'-O (3-thiotriphosphate) (ATPγS), [26] the homologous sequence Cy3-ssDNA in dsDNA will be recognized in a homologous pairing step.[24-26] Subsequently, the fluorescent stable triple-stranded DNA structures form and finally release from GO surface, resulting in restoration of dye fluorescence.



Scheme 1. Schematic illustration of GO/DNA duplex-based sensing platform via the triple DNA structure formation mediated by RecA−ssDNA nucleoprotein filament in a homogeneous solution.

To realize our design, DNA duplex first assembled on GO and then the formation of triple-stranded DNA structure based on RecA protein on GO/dsDNA complex was carried out. Oligonucleotide sequences are as follows: the dye-labeled ssDNA sequence (Cy3-ssDNA) is 5'-Cy3-GAG GAC ATT

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ATT AAT AGA TGT CAA CAA TAT-3' (Cy3 = cyanine-based dye, ssDNA was also used to interact with RecA protein to form RecA-ssDNA nucleoprotein filament), the complementary target cDNA is 5'- ATA TTG TTG ACA TCT ATT AAT AAT GTC CTC -3'. Figure 1 shows the fluorescent fluorescence spectra of Cy3-dsDNA at different conditions. Obviously, a suitable quantity of GO (75 μg/mL) could efficiently quench the fluorescence of dye up to 95% (Figure 1A, curve d), indicating the strong interaction between GO and Cy3−dsDNA. While adding RecA−ssDNA nucleoprotein filaments and incubating with adenosine-triphosphate (ATP) or ATPγS, the homologous sequence Cy3−ssDNA in dsDNA would be recognized and invaded. As shown in Figure 1A, in the presence of RecA-ssDNA filaments, when incubating with ATPγS, nearly 65% fluorescence of Cy3 was observed (curve b); however, 30% fluorescence recovering occurred in the presence of ATP (curve c), while exhibiting a much lower fluorescence response in the case of ATPγS. As known, ATPγS, a poorly hydrolyzable equivalent of ATP,[24-26] was used to prevent protein dissociation by hydrolysis, ie, in the presence of ATPγS, RecA protein will form stable complexes with ssDNA, which are active in homologous pairing and promoting formation of stable triple-stranded DNA structures.[21-26] From the point of the molecular flexibility, the triplex DNA structure is much stiffer than duplex DNA, leading to the change of absorption status of fluorophore labelled DNA duplex on GO and thereby generating the fluorescence recovering.

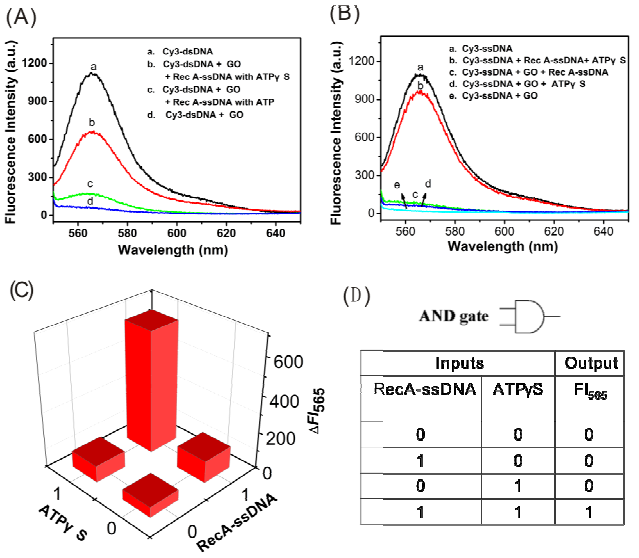

Figure 1. (A) Fluorescence responses for Cy3-dsDNA (a) in the sequential addition of GO (d), RecA−ssDNA and ATPγS (b) or RecA−ssDNA and ATP (c). (B) Fluorescence responses for Cy3-dsDNA (a) incubated with GO (e), RecA-ssDNA and ATPγS (b), GO and RecA−ssDNA (c), and GO and ATPγS (d), respectively. Concentration of Cy3-dsDNA: 20 nM; GO: 75 μg/mL; RecA protein: 1 μg/mL; ATP and ATPγS : 1 mM; Buffer: 75 mM Tris-HCl solution (pH 7.5) containing 5 mM Mg^{2+}. Excitation wavelength: 540 nm. (C) Cy3 fluorescence intensity changes at 565 nm (FI_{565}) in the form of a bar representation. (D) Truth table for the two-input AND logic gate system.

Moreover, series of conditions and optimum experiments were conducted, as shown in Figure 1B. Note, when only RecA−ssDNA filaments were added, no distinguishable fluorescence was recovered (curve c). This indicated that only the nucleoprotein filaments without ATP scarcely influenced the conformation of Cy3-dsDNA on GO, in coincidence of the fact that RecA is one kind of DNA−binding protein depending on ATP.[30] And, this also gave the evidence that Cy3-dsDNA could bind strongly on GO which would not be disassociated from GO in the presence of RecA protein. For the comparison, adding RecA-ssDNA and ATPγS into Cy3-dsDNA solution, it showed less than 10% fluorescence quenching (curve b). This could be ascribed to the fluorescence inner-filter effect of RecA protein. Moreover, when we added 1 mM ATPγS into the Cy3-dsDNA−GO solution with incubation for 2 h, no significant fluorescence enhancement was observed (curve d). From these control experiments, it could be concluded that in the presence of ATPγS, RecA−ssDNA filaments could interact with homologous dsDNA on GO and then rendered the forming of the triple-stranded DNA away from GO surface, resulting the dye fluorescence restoring.

AND logic is represented by the situation where the output of the gate occurs only when both inputs are present. As shown in Figure 1C, the RecA-ssDNA filaments–mediated fluorescence changes in the sensing system enabled the design of a DNA AND logic gate. Here the RecA-ssDNA and ATPγS were defined as the two inputs for the logic gate; Fluorescence intensity was defined as the output. Experiments were performed by testing the AND gate sensor with all four possible input combinations including (0, 0), (1, 0), (0, 1) and (1, 1). For input, the presence of RecA-ssDNA or ATPγS was defined as 1 and the absence as 0. Fluorescence here serves as the output (1 or 0). In the presence of both RecA-ssDNA and ATPγS inputs in a sample (1,1), the AND gate switches on and Cy3 fluorescence emission is produced (output 1). If one (0,1 or 1,0) or neither (0,0) of the analytes is present in the sample, no Cy3 fluorescence is produced (output 0). As regards the output, we assigned logic 1 to the situation when the fluorescence intensity was corresponding to the input (1, 1), and logic 0 to the fluorescence intensity 0.2-fold lower than that of (1, 1) (Figure 1D). This is a new concept for performance of DNA logic gates and it is the first example of a GO/DNA duplex-based RecA-ssDNA and ATPγS driven fluorescent AND logic gate.

The above findings facilitate a novel biosensing design for the detection of DNA mediated by the Rec-ssDNA nucleoprotein filaments. First, 20 nM Cy3−ssDNA was used to hybridize with variable concentration of complementary DNA (cDNA) in the presence of GO. Second, RecA−ssDNA filaments and ATPγS were added with incubation under the same conditions. Upon the reaction with RecA−ssDNA filaments, the fluorescent DNA was released from the GO surface and an increase of fluorescence intensity was achieved. Consequently, we could monitor the fluorescence intensity change corresponding to the cDNA concentration. As shown in Figure 2, the biosensing maintained a quantifiable detection range from 2 nM to 200 nM and linear detection range of 5 ~ 75 nM. According to the definition that the detection of limit (LOD) is the lowest analyte concentration required to produce a signal greater than 3 times the standard deviation of the background, the LOD for our proposed biosensing system is determined to be 1 nM. In contrast, no

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obvious fluorescence enhancement was observed when single- or double-base pair mismatched DNA was added under the same condition, indicating the high specificity to DNA sequences (Figure S1 seen in ESI).

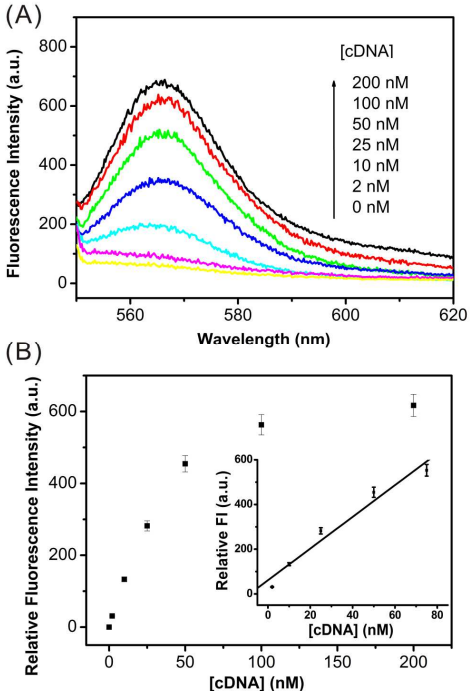

Figure 2. (A) Fluorescence spectra of Cy3-ssDNA (20 nM) hybridized with different concentration cDNA (0, 2, 10, 25, 50, 100, 200 nM) in the presence of GO and then incubated with RecA-ssDNA and ATPγS. (B) Relative fluorescence intensity (FI) over background of Cy3-ssDNA for different cDNA concentrations. Excitation wavelength: 540 nm. GO: 75 μg/mL; RecA protein: 1 μg/mL; ATPγS: 1 mM; Buffer: 75 mM Tris-HCl solution (pH 7.5) containing 5 mM Mg^{2+}.

In conclusion, we succeed in constructing a novel GO/DNA duplex-based sensing platform upon forming the triple-stranded DNA structure mediated by RecA−ssDNA nucleoprotein filaments. We found that in the presence of ATPγS, RecA−ssDNA filaments could interact with homologous dsDNA on GO and then rendered the forming of the triple-stranded DNA away from GO surface, resulting the dye fluorescence restoring. This is a new concept for performance of DNA-based fluorescent AND logic gate using the biointerface of GO/DNA duplex, which can also fluorescently detect nanomolar concentration oligonucleotide in homogeneous solution. Therefore, we hope to build upon the above the proof-of-concept experiments and investigate the promising application for detection in the real biological sample in the near future. Moreover, in comparison with previously reported graphene/ssDNA-based platforms, it is expected that the novel strategy will open new opportunities for the development and design of the graphene/DNA duplex-based systems for molecular engineering, logic gate computation and sensing applications in the future.

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Notes and references

1. Department of Chemistry, Beijing Key Laboratory for Microanalytical Methods and Instrumentation,, Tsinghua University, Beijing 100084, China. E-mail: jhli@mail.tsinghua.edu.cn.
2. Department of Optical Engineering, State Key Laboratory of Modem Optical Instrumentation, Zhejiang University, Hangzhou 310027, China
3. College of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou 310058, China

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