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Aptamer/Graphene Oxide Nanocomplex for *in Situ* Molecular Probing in Living Cells

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Abstract: Graphene has shown fascinating applications in bionanotechnology, including DNA sensing, protein assays, and drug delivery. However, exploration of graphene with intracellular monitoring and *in situ* molecular probing is still at an early stage. In this regard, we have designed an aptamer-carboxyfluorescein (FAM)/graphene oxide nanosheet (GO-nS) nanocomplex to investigate its ability for molecular probing in living cells. Results demonstrate that uptake of aptamer-FAM/GO-nS nanocomplex and cellular target monitoring were realized successfully. The dramatic delivery, protection, and sensing capabilities of GO-nS in living cells indicate that graphene oxide could be a robust candidate for many biological fields, such as DNA and protein analysis, gene and drug delivering, and intracellular tracking.

A unique ability for DNA adsorbing[1] as well as its super quenching capacity with a wide energy transfer range has shown graphene to be a robust artificial nanomaterial in bionanotechnology, with applications in DNA analysis,[2] protein assays,[3] drug delivery, *etc*.[4] Recent research developments of functionalized carbon nanotubes for macromolecule delivery,[5] semiconductor nanocrystals with unique optical properties for cell imaging,[6] and colloidal gold nanoparticles for gene regulation[7] have proved the remarkable applications of nanomaterials in many areas, from molecular biology to cellular imaging and medical diagnostics.[8] However, exploration of graphene with intracellular analysis, living cell imaging, and *in situ* monitoring still remains at a very early stage.

In this Communication, we report the demonstration of cellular delivery and *in situ* molecular probing in living cells by employing graphene oxide nanosheets (GO-nS) as DNA cargo and sensing platforms (Figure 1). Due to the particular interaction between GO and DNA molecules, an aptamer/GO-nS nanocomplex was designed and used as a real-time biosensing platform in living cell systems. An aptamer is an artificial oligonucleotide receptor derived from *in vitro* selection with high specificity and affinity for a given target.[9] Adenosine triphosphate (ATP) aptamer has been well studied for its sequence, structure, configuration, and functions in past decades due to the significance of ATP in living systems.[10] Herein, ATP−ATP aptamer recognition was carried out as a model system to elucidate whether (1) GO-nS can serve as a transporter of DNA aptamer into living cells, (2) GO-nS shows efficient protection of oligonucleotides from enzymatic cleavage during the delivery to inter- or intracellular spaces, and (3) GO-nS can act as a sensing platform with high fluorescence quenching efficiency and realize real-time target monitoring in living cells. The successful delivery to molecular targets in living cells suggest that GO could be a good vehicle to transport gene into cells, protecting the loading gene from enzymatic cleavage and enabling *in situ* molecular probing in living cells.

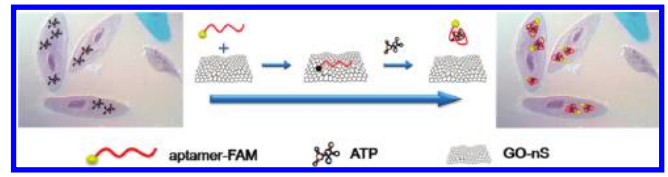

Figure 1. Schematic illustration of *in situ* molecular probing in living cells by using aptamer/GO-nS nanocomplex.

GO was synthesized by the Hummers and Hoffman method with some modifications,[11] followed by strong sonication and centrifugation to disperse and remove large GO layers. Compared with chemically synthesized ones, the as-made GO-nS were hydrophilic with a narrower size distribution (around 100 nm; see Figures S1 and S2 in the Supporting Information). To investigate the sensing performance of this aptamer/GO-nS nanocomplex, *in vitro* ATP detection was carried first. Briefly, ATP aptamer labeled with the fluorophore carboxyfluorescein (FAM) was incubated with GO-nS to form aptamer-FAM/GO-nS. After 5 min incubation, nearly 100% fluorescence quenching with very fast kinetics was observed on aptamer-FAM/GO-nS because of fluorescence resonance energy transfer (FRET) between FAM and GO-nS.[1] As we assumed, ATP aptamer could target ATP and then form the duplex configuration,[9] which would be released from the surface of GO-nS due to the weak adsorption. Consequently, extraordinary fluorescence recovery would be obtained by injecting ATP solution into the reaction system. Figure 2 displays that aptamer-FAM/GO-nS has high sensitivity and a wide detection range, from 10 μM to 2.5 mM, for *in vitro* ATP sensing. Meanwhile, excellent selectivity and affinity of this sensing platform are shown in Figures S3 and S4 in the Supporting Information. Due to the special aptamer specificity, only ATP showed obvious fluorescence recovery up to 85.7%; neither CTP, GTP, nor TTP was observed with any vivid fluorescent recovery. On the other hand, the quenching ability of GO-nS was estimated by measuring the fluorescence intensity of 100 nM aptamer-FAM solution with different amounts of GO-nS, ranging from 1 to 6 μg/mL. Here, 3 μg/mL was chosen the as optimized GO-nS concentration because fluorescence intensity was only 0.4% of the original one (Figure S5, Supporting Information). The good selectivity and sensitivity of the aptamer/GO-nS nanocomplex predicted its potential use for intracellular ATP monitoring in living cells.

After exploring the sensing property of aptamer-FAM/GO-nS *in vitro*, we further analyzed its ability for *in situ* molecular probing in living cells. For this purpose, mice epithelial cells (JB6 Cl 41-5a) were incubated with aptamer-FAM/GO-nS in growth media

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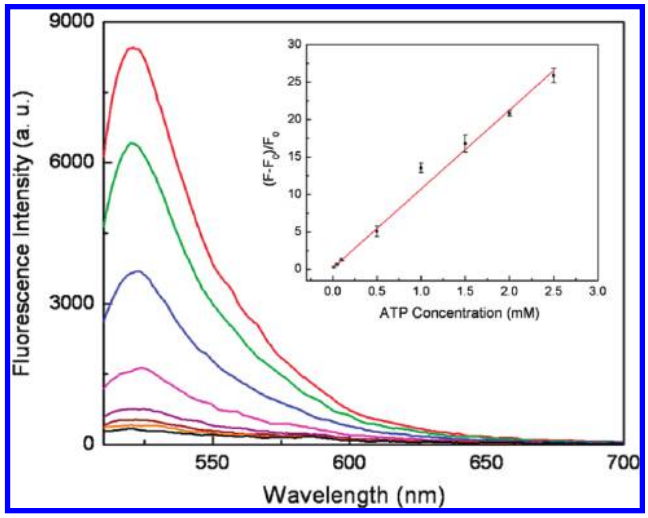

Figure 2. Fluorescence emission spectra of 100 nM ATP aptamer-FAM quenched with 3 μg/mL GO-nS (black bottom line) and fluorescence recovery by addition of ATP with concentration ranging from 10 μM to 2.5 mM (from bottom to top). Inset: linear relationship between $(F - F_0)/F_0$ (relative fluorescence intensity, where F_0 and F are the fluorescence intensity without and with the presence of ATP, respectively) and ATP concentration. Error bars were obtained from three parallel experiments.

up to 8 h at 37 °C. As shown in Figure 3, significant FAM fluorescence could be observed for JB6 cells incubated with aptamer-FAM/GO-nS (Figure 3F) after complete washing and rinsing with PBS, indicating successful intracellular aptamer delivery and ATP probing in living cells. As controls, JB6 cells were also cultured either with or without random DNA-FAM/GO-nS as well as with ATP aptamer-FAM or GO only, and almost no fluorescence signals were observed (Figure 3B,D,H,J). These results demonstrate that GO-nS is an efficient cargo for DNA transport through cell membranes.

Meanwhile, ATP aptamer loaded and transported by GO-nS could successfully target with cellular ATP to form aptamer−ATP duplex and resulted in strong fluorescence recovery. Furthermore, aptamer could also be well protected by GO-nS from enzymatic cleavage during the cellular transit. Electrophoresis by using agaros gel was carried out to demonstrate the enzymatic cleavage protection of aptamer-FAM/GO-nS. DNase was employed to simulate the enzymatic cleavage functions in living systems. Results illustrated that DNase cleavage was accessible to aptamer-FAM but inaccessible to aptamer-FAM/GO-nS (Figure S6, Supporting Information), indicating the excellent property of GO-nS to protect aptamer from enzymatic cleavage. To further confirm the uptake of aptamer-FAM/GO-nS by JB6 cells, we carried out single-cell Z-scanning (shown as Figure S7 and S8, Supporting Information), captured by wide-field and confocal microscopy.

Based on our design, as culture time elapses, more aptamer will enter into JB6 cells and then form duplexes with cellular ATP, followed by fluorescence recovery. As a result, real-time monitoring of JB6 cells cultured with random DNA-FAM/GO-nS (Figure 4A−F) and aptamer-FAM/GO-nS (Figure 4G−L) was realized using a wide-field microscope. When culture time was up to 4 h, the fluorescence intensity of JB6 cells incubated with aptamer-FAM/GO-nS increased obviously (Figure 4J) and the intensity distribution peak was shifted from 50 to 100 au. Images of JB6 cells 6 and 8 h postincubation were captured and are shown in Figure 4E,F,K,L. As one can see, fluorescence intensity of cells with aptamer-FAM/GO-nS increases as culture time elapses. However, no obvious change of fluorescence intensity was observed for the cells cultured

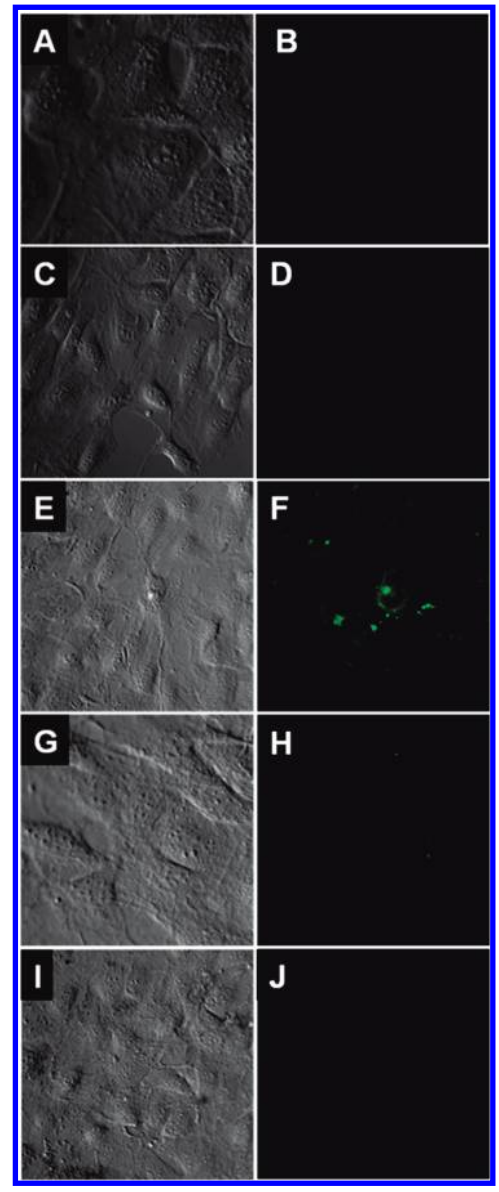

Figure 3. Cellular uptake of DNA/GO-nS hybrid shown by differential interference contrast (left) and wide-field fluorescence images (right). JB6 cells were incubated without (A,B) or with ATP aptamer-FAM (C,D), ATP aptamer-FAM/GO-nS (E,F), random DNA-FAM/GO-nS (G,H), and GO-nS (I,J) for 8 h at 37 °C. Images were obtained after extensive washing of cells with PBS.

with random DNA-FAM/GO-nS. At 8 h, the fluorescence of JB6 cells incubated with aptamer-FAM/GO-nS was brilliant and the intensity distribution peak had shifted close to 255 au. Application of aptamer-FAM/GO-nS in real-time monitoring and *in situ* cellular probing has demonstrated the promising potential of GO as a transporter and sensor element in living systems.

In summary, we have illustrated the dramatic delivery, protection, and sensing capabilities of graphene oxide nanosheets in living cells. Noncovalent binding between GO-nS and oligonucleotides along with excellent solubility and biocompatibility have shown GO-nS to be an efficient cargo and an excellent protector for cellular delivery of genes and possibly peptides or proteins in due course. Moreover, super quenching ability and the resulting FRET function suggest GO-nS as a universal sensing platform appropriate for various fluorescent probes. In conclusion, the advantages of graphene oxide shown here make it a robust candidate for use in many biological fields,

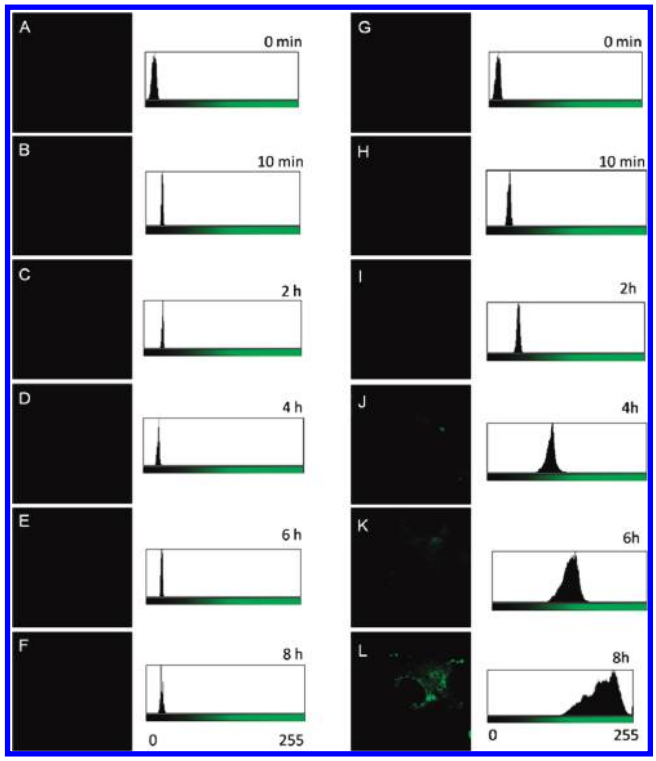

Figure 4. Real-time monitoring of JB6 cells cultured with random DNA-FAM/GO-nS (A−F) and aptamer-FAM/GO-nS (G−L) for 8 h at 37 °C. Images were captured by wide-field microscopy every 2 h, and the corresponding fluorescence density distribution with color bar is shown for each image.

such as DNA and protein analysis, gene and drug delivery, intracellular tracking as well as *in vivo* monitoring, *etc*.

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Supporting Information Available: Experimental methods and other supplementary data. This material is available free of charge via the Internet at http://pubs.acs.org.

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