Graphene Signal Amplification for Sensitive and Real-Time Fluorescence Anisotropy Detection of Small Molecules

Jinhua Liu,‡ Changyao Wang,‡ Ying Jiang,‡ Yaping Hu,‡ Jishan Li,‡ Sheng Yang,‡ Yinhui Li,† Ronghua Yang,*,† Weihong Tan,†,‡ and Cheng Zhi Huang§

†State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha, 410082, China
‡Center for Research at the Bio/Nano Interface, Department of Chemistry and Department of Physiology and Functional Genomics, Shands Cancer Center and UF Genetics Institute, University of Florida, Gainesville, Florida, 32611-7200, United States
§Education Ministry Key Laboratory on Luminescence and Real-Time Analysis, and College of Pharmaceutical Science, Southwest University, Chongqing 400715, China

ABSTRACT: Fluorescence anisotropy (FA) is a reliable, sensitive, and robust assay approach for determination of many biological targets. However, it is generally not applicable for the assay of small molecules because their molecular masses are relatively too small to produce observable FA value changes. To address this issue, we report herein the development of a FA signal amplification strategy by employing graphene oxide (GO) as the signal amplifier. Because of the extraordinarily larger volume of GO, the fluorophore exhibits very high polarization when bound to GO. Conversely, low polarization is observed when the fluorophore is dissociated from the GO. As proof-of-principle, the approach was applied to FA detection of adenosine triphosphate (ATP) with a fluorescent aptamer. The aptamer exhibits very high polarization when bound to GO, while the FA is greatly reduced when the aptamer complexes with ATP, which exhibits a maximum signal change of 0.316 and a low detection limit of 100 nM ATP in buffer solution. Successful application of this strategy has been demonstrated that it can be constructed either in a “signal-off” or in a “signal-on” detection scheme. Moreover, because FA is less affected by environmental interferences, FA measurements could be conveniently used to directly detect as low as 1.0 μM adenosine triphosphate (ATP) in human serum. The universality of the approach could be achieved to detect an array of biological analytes when complemented with the use of functional DNA structures.

Fluorescence anisotropy (FA) is a reliable and simple signaling transduction approach that provides information on molecular orientation, mobility, and interaction processes based on the change in the molecular weight of a fluorophore.1 As a result, it is under intense investigation on applications to fluorescence sensing.2,3 Aptamers are single-stranded oligonucleotides with distinct binding properties to various targets including ions, small compounds, proteins, and even cells.4,5 Using FA measurement, dye-labeled aptamers have been used for quantitative analysis of biologically related macromolecules, such as thrombin,6,7 human neutrophil elastase,8 oncoprotein PDGF,9,7 IgE,10 angiogenin,11 and lysozyme.12 The binding of target macromolecules will yield a significant change in their molecular masses and, therefore, change in the rotational diffusion rates of the labeled dyes, resulting in detectable changes in their anisotropy values. These successes, however, remain to be explored for small molecules because their molecular masses are relatively too small to produce observable anisotropy value changes.

By using an elegant design and the peculiar conformational flexibility of the aptamer, Peyrin et al. described a FA technique dedicated to detection of tyrosinamide and L-argininamide.13 Upon target binding, the designed aptamer folds from a flexible, disordered structure into a structured conformation, resulting in an anisotropy increase. For any given aptamer sequence, however, this method commonly encounters unpredictable structural alteration such that the spatial change may not induce perceptible signal transduction.14,7 For mass amplifying anisotropy detection, a competition-based probe construction has been recently developed by introducing a DNA competitor to sense the binding between the aptamer and target.15,16 The dye-labeled DNA competitor hybridizes with a partial or whole aptamer sequence but allows dehybridization when aptamer binds to the target molecule, leading to a decrease in the anisotropy signal. While this approach offers a general means of FA probe design, it can cause only a limited mass difference after target binding, which is difficult for a highly sensitive assay.
More recently, Yang et al developed a novel mass amplifying approach that consists of a targeting aptamer domain against a target molecule and a molecular mass amplifying aptamer domain for a large mass molecule such as thrombin. A target molecule binds to the probe and activates its binding ability to the anisotropy amplifier, thus significantly increasing the molecular mass and FA value of the probe/target complex. This molecular mass amplifying strategy can be used to design general aptamer probes for sensitive and selective detection of small molecules, although it needs sophisticated probe design and the use of protein as a mass amplifier. Besides biomacromolecules, nanomaterials provide an alternative means for mass amplifying the FA assay. In the recent years, molecular imprinting polymers,gold nanoparticles, and silica nanoparticles have been successfully exploited for FA-based determinations of the small species and metal ions. These methods, however, require complicated material preparation or covalent labeling of the nanoparticles with the DNA probe.

In assessing the merits and limitations of these approaches, a simple and general FA approach that can detect and quantify such small molecules with high sensitivity and selectivity are therefore urgently needed. Carbon nanoarchitectures, such as carbon nanotubes and graphene, have been demonstrated to function as both “nano-scaffold” for oligonucleotide and a “nanoquencher” of the labeled fluorophore. As a result, these carbon nanomaterials have been extensively used for fluorescent detections of nucleic acids, proteins, and small biomolecules, etc. However, applications of carbon nanomaterials to FA sensing design remain to be explored. Of particular interest, carbon nanomaterials have a large mass which, in combination with the unique DNA interactions, forms the basis of a convenient and versatile mass amplifying strategy for the FA technique. Therefore, as a continuation of our studies on fluorescent biosensor design based on carbon nanomaterials, we reported herein a new amplification strategy for the FA-based aptamer sensor (aptosensor) for detection of adenosine triphosphate (ATP) by employing graphene oxide (GO) as a mass amplifier and explore the detection scheme via either “signal off” or “signal on” detection. Compared to the analogous designs for FA assays, the present approach requires neither sophisticated probe design nor the use of biomolecule as a mass amplifier but has extraordinarily high signal amplification efficiency (anisotropy value change >0.3) and rapid response time, which has the potential for real time monitoring of a target in complicated biological environments.

**EXPERIMENTAL METHODS**

**Chemicals and Apparatus.** All oligonucleotides were prepared by TaKaRa Biotechnology Co., Ltd., (Dalian, China). They were dissolved in sterilized Milli-Q ultrapure water (18.2 MΩ) as stock solutions and were kept at −20 °C. The concentrations of the solution were estimated by UV absorption using published sequence dependent absorption coefficients. The GO used in this work was purchased from Carbon Nanotechnologies, Inc. ATP, and uridine triphosphate-(UTP), cytidine triphosphate(CTP), and guanosine triphosphate(GTP) were purchased from Aldrich (Milwaukee, WI). Other chemicals were purchased from Aldrich and were used without further purification. All work solutions were prepared with 20 mM Tris-HCl buffer solution (pH 7.4, 50 mM KCl + 10 mM MgCl2).

Fluorescence measurements were carried out on a modular spectrofluorometer (Photon Technology International Inc.)

**Analytical Chemistry**

**Fluorescence Measurements.** The working solutions of the fluorescent oligonucleotides were obtained by diluting the stock solution to about 100 nM with the Tris-HCl buffer. To study the kinetics and time dependence of the fluorescence quenching and anisotropy enhancement of the fluorescent aptamer (P1, 5′-FAM-ACCTGGGGAGTATTGCGGAGG AAGGT-3′) by GO, GO was first sonicated in doubly deionized water for 2 h to give a homogeneous black solution. After the pretreatment, an aliquot of the freshly made GO suspension (less than 1%, v/v) was added to 500 µL of Tris-HCl buffer containing 25 nM of P1 and the level of fluorescence emission intensity or anisotropy was then recorded with time. For target detection, different concentrations of ATP were first interacted with the aptamer for 5 min at room temperature and FA was then recorded with addition of an aliquot of the freshly made GO suspension.

For the anisotropy enhancement assay, the FA value of 500 µL of fluorescent DNA competitor (P2, 5′-FAM-TTCCTCCGCAATACTCCCCC-3′) and the ATP aptamer was hybridized at 38 °C for 15 min in the absence and presence of different concentrations of ATP, followed by the addition of 8.0 µg/mL GO. The level of anisotropy was then recorded with time.

**Gel Electrophoresis.** Gel electrophoresis was also carried out to confirm the binding between the aptamer and the GO or a target. The gel was prepared containing 3% agarose and 1X TBE, pH 8.0. A volume of 20 µL of different reaction products ([P1] = 1.0 µM) containing 15% glycerol were added to each lane. The gel was run at 100 V for 60 min. The running buffer also contained 1X TBE. The photograph was taken by a Bio-Rad Molecular Imager (ChemiDoc XRS + imaging system) under the UV-Trans model after exposure for 2.0 s.

**Data Analysis.** Anisotropy value (r) is a ratio, defined as the difference between the linearly polarized components of emission divided by the total light intensity, which is sensitive to changes in the rotational motion of fluorescently labeled molecules. According to the modified Perrin equation (eq 1), the r value of a rotating molecule is proportional to the viscosity of the solvent (η), size (V), and molecular mass (Mr) of the molecule,

$$\frac{1}{r} = \frac{1}{r_0} + \frac{\tau RT}{h(V + h)} \frac{1}{Mr}$$

where h is the hydration radius, T is the temperature in K, R is the molar gas constant (8.31 J/mol K), and η is the viscosity in poise. The observed r can then be calculated by eq 2,

$$r = \frac{I_{VV} - GI_{VH}}{I_{VV} + 2GI_{VH}}$$

where the subscripts V and H refer to the orientation (vertical or horizontal) of the polarizer for the intensity measurements, with the first subscript indicating the position of the excitation polarizer and the second for the emission polarizer. The G factor is defined as the following eq 3,
\[ G = \frac{I_{HV}}{I_{HH}} \]  

(3)

**RESULTS AND DISCUSSION**

**Characterization of Graphene Oxide.** To confirm the successful formation of a single layered GO sheet, the GO was analyzed by atomic force microscopy (AFM). The sheet thickness (height) of GO was approximately 1.5 nm, (Figure S1A in the Supporting Information). The chemical structure of GO was characterized by both IR and Raman spectroscopy. Several characteristic peaks of functional groups containing oxygen were observed in the IR spectrum of GO, including peaks at 1640 and 1079 cm\(^{-1}\) that resulted from C=O and C−O stretching, respectively (Figure S2A in the Supporting Information). Strong D-band absorption at 1324 cm\(^{-1}\) appeared in the Raman spectrum of GO (Figure S2B in the Supporting Information). The size distribution of GO was in the range of 50 and 200 nm (Figure S3 in the Supporting Information). The potential distribution of ζ−1 that resulted from C=O and C−O stretching, respectively (Figure S2A in the Supporting Information). Strong D-band absorption at 1324 cm\(^{-1}\) appeared in the Raman spectrum of GO (Figure S2B in the Supporting Information). The size distribution of GO was in the range of 50 and 200 nm (Figure S3 in the Supporting Information) with a ζ potential distribution of −27.3 ± 1.2 mV (Figure S4 in the Supporting Information). Energy-dispersive X-ray (EDX) spectroscopy results showed that GO included 62.98% C and 37.02% O in its structure, the atom ratio of carbon and oxygen was about 2.27, and the relative degree of oxidation was about 22.5%, which is close to the previous reported value.\(^{31}\) Taken together, these data support the premise that single-layered GO sheets were successfully prepared.

**Design of the Detection Scheme.** Our design scheme is based on the difference of binding affinity of carbon nanomaterials with single stranded (ss-) DNA and the DNA/target complex. Carbon nanomaterials have strong binding ability with ssDNA through π−π stacking interactions, but the affinity of DNA/target complex is significantly weaker than that of ssDNA.\(^{25−29}\) On the basis of the interaction difference of GO with DNA, the FA signaling can be constructed in different ways to achieve either a "signal-off" or a "signal-on" detection. In the "signal-off" detection scheme (Scheme 1A), the capture DNA was labeled with a dye. The dye-labeled ssDNA exhibits low FA in the non-Go bound state. Strong binding of the ssDNA with the GO greatly amplifies the mass of the dye, thus in turn increases the FA value. In the presence of a target, formation of DNA/target complex disturbs the interaction between the fluorescent ssDNA and GO, resulting in a decrease in the anisotropy value compared to that without a target. In the "signal-on" detection scheme (Scheme 1B), a dye-labeled ssDNA is used as a signaling transduction probe (STP). The STP is completely or partially complementary to the capture DNA to form a DNA duplex with relative low anisotropy signal. When displaced by a target, the released STP binds to GO to amplify the mass, thus leading to an increase in the anisotropy value.

As proof-of-principle, we performed FA-based detection of ATP with a fluorescein(FAM)-labeled ATP aptamer (P1)\(^{32}\) and the STP, P2. The molecular mass of ATP is 507, which is relatively too small to produce an observable change in the anisotropy value, making the FA method for detection of ATP be generally not applicable. Using GO as a mass amplifier, more than a 10-fold FA enhancement could be achieved, which provides a sensitive assay approach for ATP detection.

The feasibility of the strategy was first demonstrated by nondenaturing gel electrophoresis. As shown in Figure S5 in the Supporting Information, the free P1 displayed only one band in the PAGE gel with a strong fluorescence under UV excitation (lane a). In the presence of GO, no obvious light band can be observed (lane b), indicating the P1/GO complex is unable to penetrate into the gel at all. However, when P1 is first interacted with ATP and then mixed with GO, the reaction product exhibits a fast-moving band with observable visible light (lane c), suggesting the formed aptamer/target complex has a relative smaller mass than the P1/GO complex. These mass variations of P1 by GO and subsequently by ATP provides a basis for FA-based detection of ATP.

**Binding of P1 to GO and Anisotropy Enhancement.** A promising application of graphene in sensing technology is fluorescent detection due to its excellent capability of graphene in fluorescence resonance energy transfer (FRET).\(^{26,29}\) Graphene is a good energy acceptor in energy transfer due to its peculiar electronic properties, and the fluorescence of FAM-labeled ssDNA can be efficiently quenched by graphene due to FRET between FAM and the graphene.\(^{33,26,29}\) To characterize fluorescently the binding of P1 with GO, Figure 1 shows the real-time records of fluorescence intensity and anisotropy changes of P1 in the Tris-HCl buffer solution with 12 μg/mL GO. In Tris-HCl buffer, P1 emits strong fluorescence emission owing to the presence of the FAM-based dye. However, in the presence of GO, an obvious decrease in the P1 fluorescence emission is observed (curve a), indicating the interaction of the FAM fluorophore with GO, allowing the FRET process to occur.\(^{33}\) Figure 1 also shows the anisotropy value change of P1 under the same conditions. As expected, the FA value of the free state of P1 in the buffer is very low (0.049 ± 0.011). However, P1 undergoes a significant anisotropy increase upon addition of 12 μg/mL GO (curve b), clearly indicating that binding of GO to the ssDNA strand leads to a larger mass complex, which hindered the rotation diffusion rate of the labeled FAM. It is worth noting that the anisotropy increase of P1 reaches equilibrium within a few minutes, which has the potential for the rapid and real time monitoring of a target in homogeneous solution.

---

**Scheme 1. General Strategy for Graphene Signal Amplification Assay of Small Molecule Target with FA Reduction (A) or FA Enhancement (B) Detection**
The transition between each regime is marked with an arrow. To quantificationally evaluate the effect of GO concentration on P1 fluorescence intensity and anisotropy, real-time records of fluorescence intensity and anisotropy of P1 in the Tris-HCl buffer solution with different concentrations of GO were performed at room temperature (Figure S6 in the Supporting Information). In Figure 2, the signal changes of P1 fluorescence intensity and anisotropy, respectively, are plotted as functions of time in the presence of GO. For each measurement, the cell was filled with 500 μL of Tris-HCl buffer containing 25 nM P1, and after incubation for 2.0 min at room temperature, GO (final concentration, 12 μg/mL) was introduced into the cell. The transition between each regime is marked with an arrow.

![Figure 1](image1.png)

**Figure 1.** Real-time recordings of the fluorescence emission intensity (a) and anisotropy (b) changes of P1 as a function of time in the presence of GO. For each measurement, the cell was filled with 500 μL of Tris-HCl buffer containing 25 nM P1, and after incubation for 2.0 min at room temperature, GO (final concentration, 12 μg/mL) was introduced into the cell. The transition between each regime is marked with an arrow.

![Figure 2](image2.png)

**Figure 2.** Fluorescence quenching ($F/F_0$) and anisotropy enhancement ($\Delta r = r - r_0$) of P1 upon addition of different concentrations of GO, where $F_0$ and $F$ are the P1 fluorescence intensity in the absence and the presence of GO, respectively; and $r_0$ and $r$ are the corresponding FA value of P1. The magnitude of the error bars was calculated from the uncertainty given by three independent measurements.

![Figure 3](image3.png)

**Figure 3.** Real-time anisotropy records of P1 (25 nM) upon addition of 250 μM ATP (a) and those of 25 nM P1 upon addition of 10 μg/mL GO in the absence (b) and presence of 250 μM ATP (c). The FA values were recorded in Tris-HCl buffer solution at room temperature, and the transition between each regime is marked with an arrow.

To test whether the FA change of the P1 can be used to quantitatively detect ATP, a titration experiment was carried out in which P1 was first interacted with increased concentrations of ATP and then GO was introduced into the solution of the P1/ATP complex. As shown in Figure S7 in the Supporting Information, the magnitude of P1 anisotropy enhancement induced by GO is reduced with increasing concentrations of ATP, indicating that formation of aptamer/target complex reduces the effective binding of the aptamer with GO. In order to evaluate the response sensitivity in terms of adding to GO, Figure 4A illustrates the FA value changes of to the P1 solution produces no measurable change in anisotropy. This illustrates that the molecular mass of ATP are relatively too small to hinder the rotational diffusion rate of the FAM-labeled aptamer strand. In direct contrast, using GO as a mass amplifier, significant anisotropy reduction is observed from the P1/ATP complex. In the presence of 250 μM ATP, the FA value is changed from 0.4419 to 0.1256 and a FA value reduction of 0.3163 can be achieved. Taken together, these results indicate that the introduction of GO as the mass amplifier provides anisotropy signal amplification and improves the performance and sensitivity of the probe for detecting a small target.

To test whether the FA change of the P1 can be used to quantitatively detect ATP, a titration experiment was carried out in which P1 was first interacted with increased concentrations of ATP and then GO was introduced into the solution of the P1/ATP complex. As shown in Figure S7 in the Supporting Information, the magnitude of P1 anisotropy enhancement induced by GO is reduced with increasing concentrations of ATP, indicating that formation of aptamer/target complex reduces the effective binding of the aptamer with GO. In order to evaluate the response sensitivity in terms of adding to GO, Figure 4A illustrates the FA value changes of energy transfer and electron transfer processes. Even a small increase in the GO concentration causes a substantial energy transfer between the nanostructure and the fluorophore and thus in its fluorescence emission intensity. We attribute the increase of FA value to the results of a large molecular weight mass of the labeled FAM by forming a GO/ssDNA complex and light scattering of the nanostructure. The greater the amount of GO in the solution, the more inflexible is the fluorophore in the aqueous solution, and as a result, the FA value of P1 increased with the GO concentration even if a high concentration of GO is presented.

**ATP Detection through Anisotropy Reduction.** Results from studies demonstrate that the anisotropy signal of a fluorescent ssDNA could be greatly amplified by the complex with GO, and we envisaged that this FA increase may have potential to be used in probing the ssDNA interaction with other molecules through an anisotropy change. To demonstrate the principle-of-proof, we first examined the binding of ATP and P1 in the Tris-HCl buffer solution using FA reduction as a model. The anisotropy responses of P1 in the absence and presence of GO to ATP were monitored in real time. As shown in Figure 3, addition of a high concentration of ATP (250 μM)
P1 in the absence and presence of GO as functions of the ATP concentrations. In the absence of GO, no significant variation in FA is found in the target concentration range (trace a). In contrast, in the presence of 10 μg/mL GO, a dramatic decrease in FA is observed in the ATP concentration range of 0.2–250 μM. When the target ATP concentration is over 250 μM, the FA gradually levels off and higher ATP concentrations produce a little increase of FA. A good linear relationship between the amount of ATP and the probe’s anisotropy signal is observed at the low ATP concentration (0.5–250 μM). The limit of detection (LOD), which is calculated with the equation of $\text{LOD} = 3\sigma/k$, was estimated to be 0.12 μM, where $k$ is the slope of the calibration curve and $\sigma$ is the standard deviation of the blank solution of six measurements. Although a straightforward comparison with reported LOD is difficult because different instrumental setups and mathematical approaches have been used for their determination, we can safely state that our LOD is better than most previously reported aptamer-based fluorescent sensors for ATP. Using an elegant design or the peculiar conformational flexibility of the aptamer, a lower LOD can be achieved.

To assess the specificity of the approach, anisotropy responses of P1/GO toward ATP analogues, UTP, CTP and GTP, were examined and compared with ATP under the same conditions. Figure 4B shows that only ATP caused a largest signal upon addition of ATP. On the other hand, the GO probe partially hybridizes with STP, the complex of the aptamer with ATP displaces the STP, and the latter binds with GO, leading to an increase in in the FA signal. For signal on detection, the most important consideration is the relative stability between the aptamer/STP duplex and the aptamer/target complex. With an ideal length of the STP strand, the aptamer/STP duplex is stable enough; while, in the presence of ATP, the STP is favorable to be released from the aptamer. Because of the weak affinity between ATP and its DNA aptamer, the basis of thermodynamic prediction and the rational analysis reported in the literature, STP with 20 bp complementary sequences (P2) for the ATP aptamer was designed and was labeled with FAM at the 5′-end.

The response behavior of the system for ATP depends on the amount of GO. Fixing the aptamer and P2 concentration, when the concentration of GO was too low, the GO had little function on P2 mass amplification, resulting in a poor reporting signal upon addition of ATP. On the other hand, the GO concentration should not be too high, otherwise the background signal from the duplex/GO is expected to be high and thus in turn leads to a low response sensitivity. At a fixed P2 and aptamer concentration of 25 nM, respectively, 8.0 μg/mL GO was optimized to turn out a better sensitivity (Figure S8 in the Supporting Information).

With the optimized conditions, FA measurements were performed in Tris-HCl buffer solution. In the experiment, 25 nM STP and the aptamer was hybridized at 38 °C for 30 min in the absence and presence of different concentrations of ATP, followed by the addition of 8.0 μg/mL GO. The level of anisotropy was then recorded with time. Figure S9 in the Supporting Information shows the FA value of P2 observed upon adding different concentrations of ATP. With the increase of ATP concentration, the resulting FA increases due to competitive binding of ATP and the STP with the aptamer and, as a result, an increase of the concentration of free STP to bind.
with GO. Trace c of Figure 4A illustrates the FA value change of P2 as a function of the ATP concentrations. A dramatic change in FA is observed in the ATP concentration range of 2.0–50 μM. We reasoned that the sensitivity could be further improved by redesigning the competitor sequence and using optimal dye or nanomaterials. The specificity of the detection system is tested by introduction of nonspecific target GTP. The result is a nominal signal change compared to that of the specific target ATP with same concentration (data not shown), thus demonstrating a high degree of specificity.

**Quantitative Detection of ATP in Human Serum.** As the experimental data show, our proposed approach works well for ATP assay in relative simple and pure buffer systems with excellent sensitivity and selectivity. On the other hand, ATP commonly exists in complex biological samples. For example, the concentration of human serum ATP is roughly 1000 nM level, detection of ATP concentration in human serum is the clinical requirement.

Therefore, a further application challenge of an assay method is to tolerate any interference from complex biological samples. Differing from the steady-state fluorescence intensity measurement, which differentiates the fluorescence signals between the probe and background species is often difficult due to ubiquitous endogenous fluorescent components of the species, FA has the advantage of being less affected by environmental interferences, making that technique be used for complex biological samples. To evaluate the potential effectiveness of our approach in bioassays, P1/GO was tested for quantitative detection of ATP in human serum through FA reduction. Surprisingly, controlled experiment suggests that the addition of diluted serum to the Tris-HCl buffer solution of P1 significantly enhances the FA signal observed, and no reliable FA change can be achieved upon addition of a high concentration of ATP (Figure 5A). This FA increase is probably due to nonspecific bindings of the ssDNA strands with biomolecules in diluted serum. It is known that more than 10 abundant proteins (about 90% of total protein in amount) are presented in human serum. Interestingly, if P1 was first interacted with diluted serum spiked with GO, the FA maintains its sensitivity to the ATP and shows FA reductions similar to that obtained in a standard buffer system (Figure 5A). Upon addition of 100 μM ATP to the P1/GO diluted serum solution, the FA value was reduced from 0.253 to 0.116. This observation may suggest that the formation of the ssDNA/GO complex can overcome the possible interferences resulting from nonspecific binding and thus high FA response of the human serum itself.

To demonstrate the feasibility for a quantitative ATP assay, Figure S10 in the Supporting Information shows the real-time FA records of P1/GO in 20% human serum with various concentrations of ATP. When the concentration of ATP increased, the anisotropy signal decreased accordingly. A dramatic reduction in FA was observed in the ATP concentration range of 1.0 to 100 μM. In Figure 5B, the FA reduction, Δr (Δr = r − r0) is plotted as a function of the ATP concentration, where r0 and r are the FA values of P1/GO in the absence and the presence of ATP, respectively. The resulting Δr value is proportional to the ATP concentrations from 1.0 μM to 50 μM, demonstrating the efficiency of the approach for direct quantification of ATP in complex biological fluids.

# CONCLUSION

On the basis of different affinities of the DNA aptamer and the aptamer/target complex with carbon nanostructure, a novel signal amplifying strategy to construct an anisotropy aptosensor for small biomolecule detection has been developed. The proposed approach can be engineered in ways that offer unique advantages and capabilities that are not available from conventional molecular systems and nanomaterial-based biosensors. First of all, the design makes it possible to construct FA sensors against small molecules. The FA technique presented here measures a signal that is neither influenced by the detection system nor dependent on conformational changes of the aptamer. Second, GO shows high anisotropy amplification efficiency with the enhanced FA value of more than 0.5. This large enhancement of anisotropy avoids signal interference resulting from nonspecific biomolecule binding and thus greatly improves the sensitivity for the detection. Finally, and most important, although we have demonstrated here the detection of ATP only, significantly this method can in principle be used to detect different analytes, including small molecules, metal ions, or nucleic acids, by substituting the ATP aptamer with synthetic artificial bases that selectively bind the other analytes. These features establish the universality and simplicity of the platform and could, therefore, provide the groundwork for the design of other nanodevices for biosensing applications.
The work was supported by National Natural Science Foundation of China (Grants 21075032, 21005026, and 21135001), the “973” National Key Basic Research Program (Grant 2011CB91100-0), and the special fund of Chongqing Key Laboratory.

REFERENCES

(7) Zhang, D. P.; Zhao, Q.; Zhao, B. L.; Wang, H. L. Anal. Chem. 2012, 84, 3070−3074.
(20) Ye, B. C.; Yin, B. C. Angew. Chem., Int. Ed. 2008, 47, 8386−8389.