약학석사학위논문

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서울대학교 약학대학원

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지도교수 오 유 경

이 논문을 약학석사학위논문으로 제출함

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**2014년 12월**

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# Abstract

# Graphene Nanosheets for Target Detection

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Here, we report a double stranded and dual anchored fluorescent aptamer on reduced graphene oxide (rGO) for sensitive and selective detection of interferon-gamma (IFN-γ) in biological samples. The aptasensor is composed of IFN-γ specific fluorescent aptamer with BHQ1 as one anchoring moiety, which forms double stranded sequences with complementary oligonucleotide sequence with BHQ1 as the other anchoring moiety, and rGO nanosheets. The double stranded and dual anchored fluorescent aptamer on rGO (AptasensorGO) showed 7.3 and 4.7-fold higher fluorescence intensity compared to single stranded and single anchored fluorescent aptamer on rGO, and double stranded and single anchored fluorescent aptamer on rGO, respectively. The fluorescence intensities of AptasensorGO were influenced by the length of DNA aptamer sequences, showing the highest intensity at 36 bp. AptasensorGO was specifically sensitive to IFN-γ in buffer and human

serum. AptasensorGO detected IFN-γ in buffer and human with linearity ranges over 5 orders of magnitude (from 100 pg/ml to 10 μg/ml), showing the regression coefficient of 0.9982 and 0.9838, respectively. Moreover, AptasensorGO showed fluorescence intensity changes to IFN-γ, but not to non-target proteins such as interluekin-2 and tumor necrosis factor-α. Only after 30 min of incubation with AptasensorGO, the levels of IFN-γ in human immunodeficiency virus-positive patient plasma samples were quantified to range from 250 to 500 ng/ml. Taken together, the nano-rGO platform of double stranded and dual anchored fluorescence aptasensor provides new opportunities for detection of cytokines such as IFN-γ and could be applied to rapidly monitor the cytokine levels in human patient samples.

**Keywords:** Nano detectors, Graphene nanosheets, Aptamers, Rapid detection, Target-specificity, Target-sensitivity

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# Ⅰ. Introduction

Aptamers are short, single-stranded DNA (ssDNA) or RNA oligonucleotides that can bind a wide range of targets, including proteins [1], DNA [2,3] small molecules [4], and metal ions [5] with high specificity and affinity. There has been increased interest in the development of aptamer-based sensors because of the advantages they offer in terms of high thermal stability, ease of chemical modification, and capability of incorporating surface-binding or sensing moieties compared to antibodies [6,7]. a number of detection strategies, including fluorescence, surface-enhanced Raman spectroscopy, microgravimetry, and electrochemistry. Among these approaches, detection by monitoring changes in fluorescence is an attractive option owing to features such as high sensitivity, reproducibility, and facile operation [8,9]. A conventional aptamer-based sensor detects fluorescence resonance energy transfer signals when a ligand-induced conformational change in a fluorophore- and quencher-labeled aptamer occurs. However, the sensitivity of such system is influenced considerably by the length of quencher-labeled DNA and temperature [10]. Thus, there remains a need for the development of new platforms that can selectively and sensitively detect target molecules.

Graphene, a two-dimensional nanosheet, has recently received considerable attention because of its remarkable electronic, mechanical, and thermal properties [11]. Reduced graphene oxide(rGO) can be chemically synthesized by placing graphene oxide(GO) in a solution of hydrazine [12]. Since rGO contains more crystalline graphene regions on the sheet than GO, aptamers

adsorb more strongly onto a rGO surface through hydrophobic and π-π stacking interactions between the ring structures  nucleobases [13]. Moreover, rGO can act as a more effective distance-dependent fluorescence quencher than GO or graphene [14]. This combination of high fluorescence quenching efficiency and superb ssDNA adsorption ability makes rGO suitable for application to the detection machinery of aptamer-based nanodetectors.

Cytokines are often secreted by immune cells in response to various pathogens [15]. Monitoring secreted cytokines can provide diagnostic information about various infectious diseases in patients. For example, in human immunodeficiency virus (HIV)-infected patients, T-helper and cytotoxic T-lymphocytes vigorously produce the cytokine protein such as interferon-γ (IFN-γ), resulting in low viremia and slow progression of the disease [16, 17]. It is therefore important to detect cytokine levels for accurate clinical diagnosis. Conventional antibody-based immunoassays, such as enzyme-linked immunosorbent assays, are common methods for detecting and quantifying secreted cytokines. Although immunoassays are sensitive and specific for the target protein, they require multiple washing steps, several hours of reaction time, and the use of expensive reagents [18]. The development of a new assay system that save time and reduce costs would thus be desirable.

In this study, we designed a double-stranded, dual-anchored, fluorescent (FAM-labeled) aptamer on rGO nanosheets for quantitation of target protein in biological samples. To prevent non-specific quenching of fluorescent aptamer upon interaction with rGO, we hybridized the fluorescent aptamer with a complementary sequence to form double strands. To fortify the anchoring on rGO, we labeled the double-stranded fluorescent aptamer with

two anchoring moieties. As a model target protein specific for the aptamer, IFN-γ was used.

# Ⅱ. Materials & Methods

## 2. 1. Synthesis of rGO nanosheets

GO was prepared from graphite powder following a modified Hummer's method [12]. Briefly, graphite powder (0.5 g; Sigma-Aldrich, St. Louis, MO, USA) was added to cold $H_2SO_4$ (23 ml).While this mixture was gradually stirred on ice, $KMnO_4$ (3 g) and $NaNO_3$ (0.5 g) were added slowly. The resulting mixture was further stirred for 1 h at 35 ℃. Subsequently, 46 ml of triple-distilled water (TDW) was added and the mixture was incubated at 90°C for 1 h. The reaction was halted by adding 140 ml of TDW and 10 ml of 30% H2O2. The reaction product was washed and purified by repeated centrifugation, first with an aqueous 5% HCl solution and then with TDW (three times). Finally, the product was suspended in TDW and sonicated for 2 h to exfoliate the GO layers into GO nanosheets. Unexfoliated GO was removed by centrifugation at 1600 x g for 10 min. The supernatant containing GO nanosheets was collected and filtered through 0.2-mm polycarbonate membrane filters (Millipore Corp., Billerica, MA, USA) using an extruder (Northern Lipid, British Columbia, Canada).

GO nanosheets were subsequently reduced to generate rGO nanosheets according to the method Li and colleagues [19], with slight modification. Briefly, 2.0 ml of homogeneously dispersed GO nanosheet solution was mixed with 8.0 ml of TDW, 0.5 ml of ammonia solution (28 wt% in water; Junsei Chemical Co., Tokyo, Japan), and 5.0 ml of hydrazine monohydrate (64% in water). The resultant mixture was stirred in a water bath (80°C) for

10 min, and then removed from the water bath and allowed to cool to room temperature. Excess hydrazine and ammonia were removed by dialyzing the mixture (MWCO 100K; Spectrum Laboratories, Inc., Rancho Dominguez, CA, USA) with TDW for 2 d with four changes of TDW over a 12-h interval. The obtained rGO nanosheets dispersed in TDW were stored at 4°C until use. The final concentration of the prepared rGO was 1 mg/ml.

## 2. 2. Anchoring of fluorescent aptamer on to rGO nanosheets

Double-stranded aptamer structures dually anchored with Black Hole Quencher-1 (BHQ1) were designed for perpendicular anchoring of IFN-γ–specific fluorescent ssDNA aptamers (36 bp) to rGO nanosheets (Fig. 3A). Single-stranded IFN- γ–specific fluorescent DNA aptamers (ssAptamer, 25 μg/ml, Bioneer, Daejeon, South Korea) containing FAM dye at the 5'-end and BHQ1 at the 3'-end were hybridized in hybridization buffer (10 mM Tris-HCl, 1 mM EDTA, 100 mM NaCl, pH 8.0) with complementary ssDNA oligonucleotides singly labeled with BHQ1 (25 μg/ml). Double-stranded fluorescent aptamers (dsAptamer) were formed by heating the solution containing ssAptamers of various lengths and complementary oligomers to 95°C for 5 min and slowly cooling to 30°C to allow hybridization. Double-stranded, dual-anchored, fluorescent aptamers (dsDA-Aptamer; 50 μg/ml) were immobilized onto the rGO surfaces by adding 10 μg/ml of rGO to the resultant solution. In some experiments, ssAptamers were hybridized with complementary DNA sequences with or without BHQ1, to which 10 μg/ml of rGO was added. The mixture was vortexed vigorously and incubated at room temperature for 30 min to induce formation of

dsDA-Aptamers on rGO nanosheets to yield Graptasheet. The solution was centrifuged at 6,000 x g for 2 min to remove excess, unattached aptamers. The supernatant was discarded and the remaining pellet was suspended in hybridization buffer (200 μl).

## 2. 3. Target protein sensitivity and specificity tests

The sensitivity of Graptasheet for its target protein was evaluated by incubating 10 μg/ml of Graptasheet with different concentrations of human IFN-γ (R&D Systems, Inc., Minneapolis, MN, USA) ranging from 100 pg/ml to 10 μg/ml. Specificity was demonstrated by challenging Graptasheet with 10 μg/ml of human tumor necrosis factor-α (TNF- α; R&D Systems), interleukin-2 (IL-2; R&D Systems), or the analyte of interest (1 μg/ml of IFN-γ. After incubating for 3 min at room temperature, the solution was centrifuged at 6,000 x g for 2 min to remove any excess, unreacted materials and the remaining pellet was resuspended in hybridization buffer (200 μl). The binding of IFN-γ to Graptasheet was determined by exciting at a wavelength of 485 nm and measuring fluorescence intensity at an emission wavelength of 520 nm using a spectrophotometer (Molecular Devices, Sunnyvale, CA, USA).

## 2. 4. Ex vivo detection of target protein in human serum

To detect IFN-γ in human serum using Graptasheet, we spiked serum samples from healthy individuals (Scipak Ltd, Kent, UK) with different

concentrations of IFN- γ (100 pg/ml to 10 μg/ml) and then added Graptasheet (10 μg/ml) to each spiked serum sample. After incubating for 3 min at room temperature, the solution was centrifuged at 6,000 x g for 2 min to remove any excess, unreacted materials and the remaining pellet was resuspended in hybridization buffer (200 μl). The concentrations of IFN- γ in human serum were determined by exciting at a wavelength of 485 nm and measuring fluorescence intensity at an emission wavelength of 520 nm using a spectrophotometer (Molecular Devices, Sunnyvale, CA, USA).

## 2. 5. Detection of target protein in human patient samples

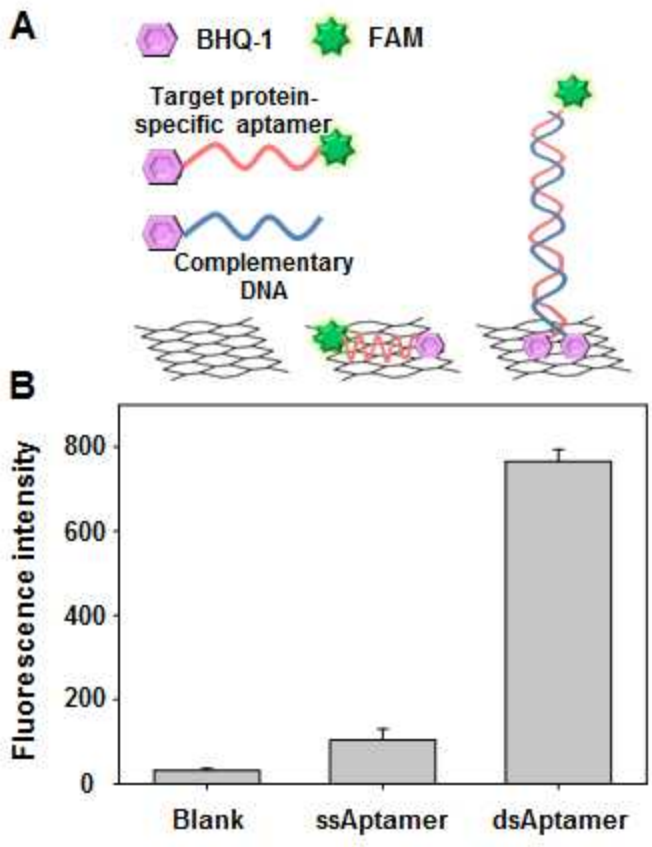
Graptasheet was used to detect IFN- γ in serum samples from HIV-positive patients. Solutions were prepared by adding Graptasheet (10 μg/ml) to 120 μl of phosphate-buffered saline (PBS) and 80 μl of serum samples from healthy individuals (Scipak Ltd, Kent, UK) or HIV-positive patients (Korea National Public Health Institute, Osong, Republic of Korea). Incubation and processing of samples, and determination of fluorescence intensity, were as described above.

# Ⅲ. Results

## 3. 1. Effect of the double-stranded structure of fluorescent aptamer on evasion of fluorescence quenching by rGO

The double-stranded structure of fluorescent aptamers played a major role in preventing the severe quenching of fluorescence intensity by associated rGO nanosheets. To test the effect of the structure of FAM-labeled, fluorescent aptamers on fluorescence quenching by rGO, we anchored ssAptamers or dsAptamers on rGO nanosheets (Fig. 1A) and compared fluorescence intensity. The fluorescence intensity of rGO alone was negligible. The fluorescence intensity of ssAptamers was significantly quenched loading onto rGO nanosheets. In contrast, dsAptamers showed higher retention of fluorescence after complexation on rGO. The fluorescence intensity of dsAptamers on rGO was 7.3-fold higher than that of ssAptamers on rGO (Fig. 1B).

**Figure 1. Fluorescence intensity of ssAptamers and dsAptamers on rGO.**

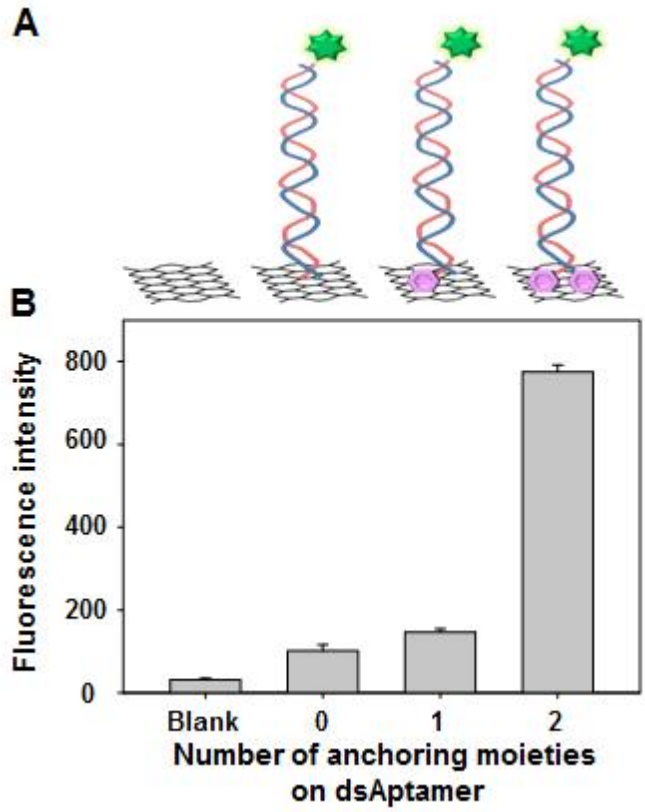



(A) Schematic representation of ssAptamers and dsAptamers anchored on a rGO nanosheet. (B) Fluorescence intensities of rGO blank, ssAptamers on rGO, and dsAptamers on rGO were determined at an excitation wavelength of 485 nm and an emission wavelength of 520 nm (n=4).

## 3. 2. Effect of the number of anchorages on dsAptamer

For the proposed aptamer-based nanodetectors to function as envisioned, dsAptamers must be able to firmly attach to rGO surfaces without a loss of fluorescence. To this end, we formed dsAptamers containing 0, 1, or 2 BHQ1 anchoring moieties and tested their retention of fluorescence upon anchoring to rGO. As illustrated in Fig. 2A, dsAptamers containing 1 or 2 BHQ1 moieties were prepared by hybridizing BHQ1-containing ssAptamers with a complementary strand containing or lacking BHQ1. Hybridization of ssAptamers without BHQ1 with a complementary strand without BHQ1 yielded dsAptamers with no BHQ1 moiety. Upon loading onto rGO, dsAptamers with 0 or 1 BHQ1 anchoring moiety failed to show a distinctive recovery of fluorescence. In contrast, dsAptamers containing 2 BHQ1 anchoring moieties (dsDA-Aptamers) retained their fluorescence intensity upon binding to rGO (Fig. 2B). Thus, a critical element in the proposed aptamer-based nanodetectors design is the presence of dual anchoring moieties.

**Figure 2. Fluorescence intensity on rGO nanosheets of dsAptamers containing varying numbers of BHQ1 anchoring moieties.**

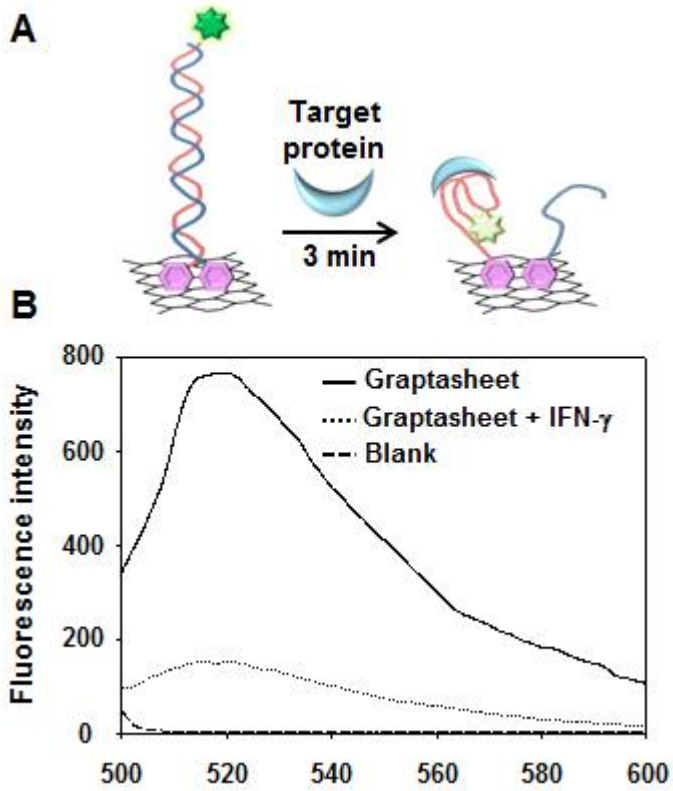



(A) Illustration of dsAptamers containing 0, 1, or 2 BHQ1 anchoring moieties attached to rGO. (B) dsAptamers differing in the number of anchoring moieties were incubated for 30 min with rGO. After removal of unattached dsAptamers, the fluorescence intensity of each dsAptamer on rGO was measured at an emission wavelength of 520 nm (n=4).

## 3. 3. Detection of target protein using Graptasheet

Based on the results shown in Fig. 1B and Fig. 2B, a dsDA-Aptamer on rGO nanosheets, designated Graptasheet, was chosen for subsequent experiments. Graptasheet was constructed by hybridizing an ssAptamer (shown in red) possessing a FAM on the 5'-end and a BHQ1 on 3'-end with a complementary oligomer (shown in blue) containing a BHQ1-modified 5'-end. In application, Graptasheet monitors specific binding to its target, in this case IFN-γ, a model protein, by converting from an unbound, fluorescent state ("on") to a target-bound, fluorescence-quenched state ("off"). This is presented schematically in Fig. 3A, which shows that as IFN- γ approaches and interacts with the IFN- γ–specific, fluorescent dsDA-Aptamer, the aptamer strand binds the target protein IFN- γ, which effectively displaces the double-stranded structure into two single strands. As a consequence, the two denatured strands, together with the fluorophore, become proximate to the rGO surface. The loss of distance between the fluorophore and the rGO surface due to IFN- γ binding quenches the fluorophore, result in an "off" signal. To evaluate the displacement of dsDA-Aptamer by IFN- γ, we measured the fluorescence emission intensity of FAM from dsDA-Aptamers attached to rGO before and after treatment with IFN- γ. rGO alone in buffer was used as a blank. As shown in Fig. 3B, the fluorescent intensity of Graptasheet decreased by 2.8-fold following exposure to 1 μg/ml of IFN- γ.

**Figure 3. Rapid changes in fluorescence intensity of Graptasheet in the presence of target protein.**

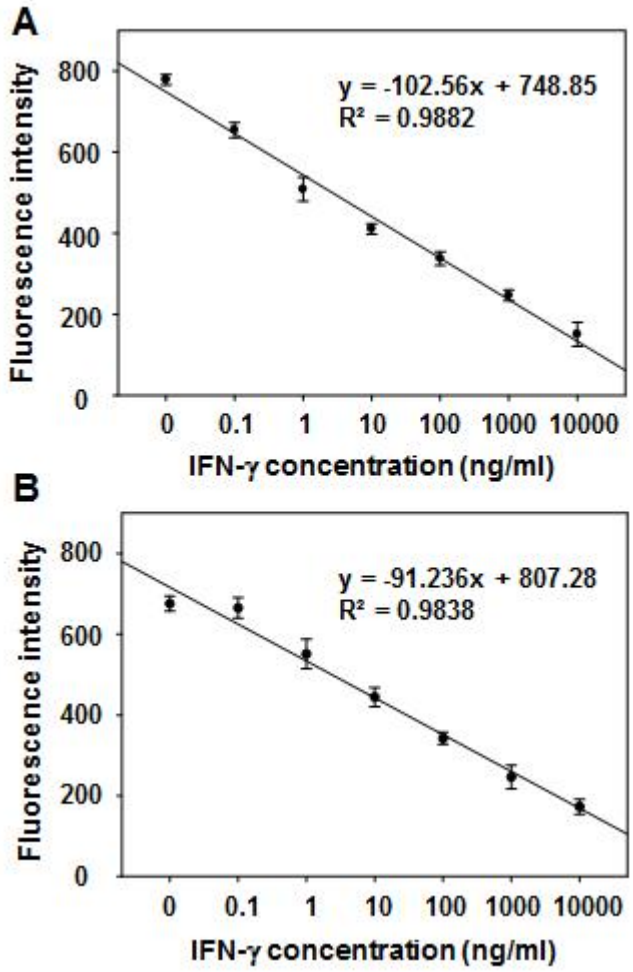



(A) Schematic illustration of the fluorescence assay for target protein detection using Graptasheet. (B) Graptasheet (10 μg/ml) was incubated for 3 min with 1 μg/ml of a model target protein, IFN-γ. After centrifugation, the pellets were resuspended and the fluorescence intensity was measured over emission wavelengths of 500-600 nm.

## 3. 4. Sensitivity of Graptasheet for target protein in buffer and human serum

To evaluate the utility of the proposed aptamer-based nanodetectors as a quantification method for target proteins under physiological conditions, we incubated Graptasheet with samples of buffer or human serum spiked with different concentrations of IFN- γ. As shown in Figure 5a, the change in the fluorescence signal of Graptasheet upon binding IFN- γ in buffer was linear from 100 pg/ml to 10 μg/ml. The regression coefficient of the trend-line equation was 0.9882 (Fig. 4A). Similar to IFN- γ in buffer, IFN- γ in human serum exhibited fluorescence intensity changes that were linear from 100 pg/ml to 10 μg/ml, with a regression coefficient of 0.9838 (Fig. 4B).

**Figure 4. Linearity in target protein detection by Graptasheet in PBS and human serum.**

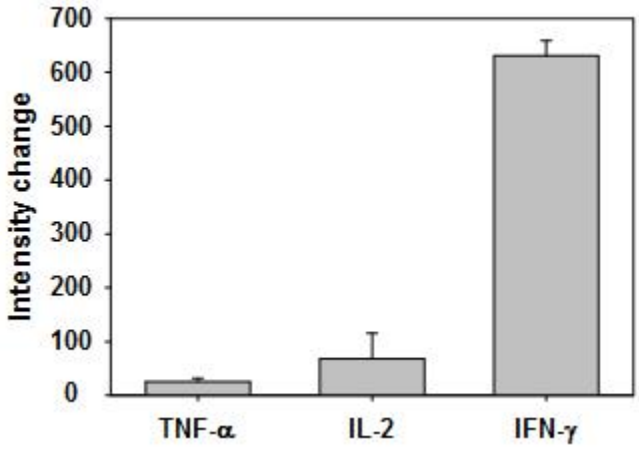



Graptasheet (10 μg/ml) was incubated for 3 min with PBS (A) or healthy human serum (B) spiked with different concentrations of IFN-γ. After incubation, the mixture was centrifuged for 2 min and the resuspended pellets were spectrophotometrically measured at an emission wavelength of 520 nm (n=5).

## 3. 5. Selectivity of Graptasheet for target protein

Graptasheet exhibited specificity for its target model protein, IFN- γ. To test the specificity of Graptasheet, we measured the degree of fluorescence intensity change after challenge with target or non-target proteins (Fig. 5). After treatment of Graptasheet with 10 μg/ml of IL-2 or TNF-α, there was no apparent change in fluorescence. In contrast, a significant change in fluorescence intensity was observed after treatment of Graptasheet with 1 μg/ml of IFN- γ.

**Figure 5. Changes in the fluorescence intensity of target protein-specific aptamer-anchored Graptasheet in response to different proteins.**

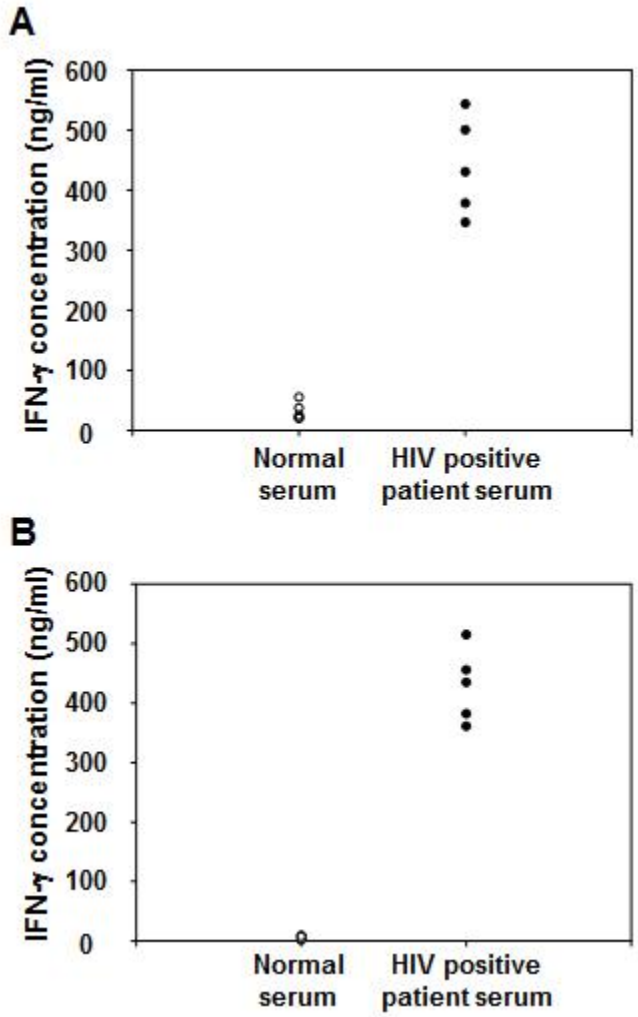



Graptasheet (10 μg/ml) was incubated for 3 min with the target protein, IFN-γ 1 μg/ml, or non-target proteins such as IL-2 (10 μg/ml) and TNF-α (10 μg/ml) in PBS. After incubation, the mixture was centrifuged and the resuspended pellets were spectrophotometrically measured at an emission wavelength of 520 nm (n=4).

## 3. 6. Graptasheet-based detection of target protein in patient samples

For clinical application of this system, we investigated the ability of Graptasheet to detect target protein in patient serum samples. Upon addition of healthy human serum samples to Graptasheet, there was little change in fluorescence intensity (Fig. 6A). In contrast, the addition of HIV-positive human serum samples (n=5) resulted in a significant change in the fluorescence intensity of Graptasheet. The concentrations of IFN-γ in all HIV-positive patient samples were quantified by reference to the standard curve prepared from IFN-γ–spiked human serum samples and were determined to range from 330 to 550 ng/ml. To evaluate validity of the proposed biosensor, ELISA analysis (Quantikine ELISA Human IFN-γ, R&D Systems, INC., USA) was performed as a standard assay for IFN-γ detection. Values obtained from ELISA analysis (Fig. 6B) revealed good correlation between the proposed assay and ELISA, thereby confirming possibility of practical utilization of the proposed assay.

**Figure 6. Quantification of target protein levels in patient serum samples.**




Practicality of the biosensor evaluated. (A) Graptasheet (10 μg/ml) was incubated for 3 min with serum samples from normal or HIV-positive patients then pectrophotometrically measured at an emission wavelength of 520 nm. The concentration of IFN-γ was calculated in reference to a standard curve (n=5). (B) The same serum samples from normal or HIV-positive patients analyzed with the IFN-γ ELISA kit.

# Ⅳ. Discussion

Here, we report the development of a dsDA-Aptamer and rGO-based nanodetectors, termed Graptasheet, that is capable of rapid detection and quantification of target protein in biological samples (human serum) with high sensitivity and specificity. These studies established the physicochemical factors that are important in the design of Graptasheet: a double-stranded aptamer structure, the presence of two anchoring moieties in the dsDA-Aptamers, and dsDA-Aptamer length.

The double-stranded structure of dsDA-Aptamers was determined to be critical for fluorescence-based target detection by Graptasheet. Since the nucleobases of dsDNA are shielded within the negatively charged phosphate backbone, the occurrence of π-π stacking interactions between graphene and nucleobases decreases dramatically [20]. A rigid duplex dsDA-Aptamer therefore cannot be adsorbed onto the rGO surface, resulting in the maintenance of strong fluorescence intensity (Fig. 1B).

For anchoring of dsDA-Aptamers onto rGO nanosheets, the presence of two BHQ1 anchoring moieties, one each in the aptamer and the complementary oligomer, was found to be essential. Only the dsDA-Aptamer containing two BHQ1 moieties showed significant fluorescence intensity; dsAptamers with no or one BHQ1 moiety showed no significant fluorescence upon rGO binding (Fig. 2B). It has been reported that rGO nanosheets are involved in π-π stacking interactions with molecules that possess aromatic rings [21]. A BHQ1 molecule contains three aromatic rings, which consequently results in strong π-π stacking interactions with rGO. It has been

reported that a fluorophore with a chromophore-like structure similar to that of BHQ1 was able to bind to single-walled carbon nanotubes by triggering π-π stacking interaction involving its aromatic fluorescein isothiocyanate domain [22]. Inclusion of two BHQ1 moieties in dsDA-Aptamers was therefore required to enhance binding to rGO surfaces.

We observed a reduction in the fluorescence intensity of Graptasheet upon addition of a model target protein IFN-γ, demonstrating the utility of Graptasheet as a probe for detecting the presence of the target protein. Owing to the greater affinity of the ssAptamer for IFN- γ compared to that of the complementary oligomer strand, the target protein binding resulted in the formation of ssAptamer-target protein complex, the dissociation of dsDA-Aptamers, and fluorescence quenching proportional to the amount of the target protein in the samples. Indeed, surface plasmon resonance studies have demonstrated that target protein binding to an aptamer displaces dsDNA into ssDNA [10]. Upon formation of an ssAptamer-target protein complex, the FAM moiety of the complex comes in close proximity to the rGO surface and thereby enters the effective quenching range of rGO, leading to a considerable decrease in fluorescence intensity.

Sensitivity over a wide dynamic range is an important characteristic of a biosensor. Graptasheet showed a five-orders-of-magnitude linear dynamic range, with a limit of detection of 0.1 ng/ml (Fig. 4). A previous study reported that a gold substrate-anchored electrochemical aptamer biosensor recognized the target protein over a range of 1 to 160 ng/ml [23]. The higher sensitivity of Graptasheet compared to the aptamer-based electrochemical biosensor might be attributable to the fluorophore-based

fluorometric detection principle of dsDA-Aptamers. Previously, a graphene-based sensor was designed for the detection of thrombin [24]. In this latter study, the graphene-based sensor showed a linear response over a concentration range of two orders of magnitude. The wide dynamic range of Graptasheet is like due, in part, to the strong quenching effect of rGO, which broadens the intensity difference compared to the unquenched intensity. A recent study reported that rGO is a better quencher for adsorbed fluorophores than GO and graphite, and exhibits a high quenching efficiency for various fluorophores ranging from green-, red-, and far-red-emitting dyes to quantum dots [25,26].

In addition to sensitivity, specificity is a crucial component of a biosensor. We found that Graptasheet showed specificity for the target model protein, IFN-γ, as evidenced by the absence of a fluorescence change in the presence of TNF-α or IL-2 (Fig. 5). This specificity of Graptasheet is conferred by the IFN-γ–specific aptamer sequence used in the dsDA-Aptamer. The nucleic acid sequence of the IFN-γ–specific aptamer has been previously reported [27]. In addition, modifications to aptamer sequences, such as extra DNA sequences, biotin, or polyethylene glycol, have been reported to have no effect on the affinity between IFN- γ and aptamer [10,23]. These previous findings are consistent with our observation that the modified ssAptamer sequences used in Graptasheet retained their affinity for the target protein.

Using Graptasheet, we were able to quantify the levels of target protein in serum from patients (Fig. 6). It has been reported that the levels of various cytokines are elevated in the blood of HIV-infected patients [16,17]. HIV-patient serum sample was thus chosen to test the feasibility of

Graptasheet for clinical application. Our results demonstrate that Graptasheet has sufficient specificity and sensitivity for use on clinical samples. The specific detection of target protein using Graptasheet provides a basis for concluding that a rGO-based fluorescent aptamer-based nanodetectors nano-platform could not only be applied to monitor or diagnose infectious diseases, such as tuberculosis and HIV, it could also be used in biochemical studies to analyze cellular secretion of specific cytokines. Although in this study we used IFN-γ as a target model protein in human patient samples, the Graptasheet concept could be broadly applied to detect other chemicals and cytokines by replacing the aptamer sequences with other target-specific aptamers.

From an industrial perspective, Graptasheet has advantages in terms of processing time and costs compared to conventional cytokine assay systems. Unlike immunoassays which required at least a few hours to complete, the detection of target protein in human patient samples using Graptasheet required only three steps and took less than 10 min to complete, including 3 min for incubation with human serum samples, 2 min for centrifugation, and 1 min for fluorometric detection. Because Graptasheet is composed of rGO, a FAM- and BHQ1-labeled DNA aptamer sequence and complementary oligomer, the cost is expected to be lower than that of antibody-based assay systems. Moreover, the higher stability of rGO and DNA compared to antibodies may provide a longer storage time for Graptasheet.

# Ⅴ. Conclusion

In this study, we developed Graptasheet, a dsDA-Aptamer and rGO-based nanodetectors for speedy and specific detection of the target protein in biological and clinical patient samples. Detecting target protein by monitoring changes in fluorescence intensity of a FAM-labeled aptamer that occur upon target binding required minimizing the quenching of FAM fluorescence upon complexation of the aptamer with rGO. This was accomplished by forming a double-stranded structure, and introducing two anchoring moieties. Graptasheet showed specificity and sensitivity to target protein in serum samples over a five-orders-of-magnitude dynamic range, and was capable of rapidly measuring the concentrations of target protein in patient serum samples with only less than 10 min for whole process. Collectively, our results demonstrate that an rGO-based fluorescent aptamer-based Graptasheet nanodetectors could have a wide spectrum of applications for monitoring various target proteins in human clinical samples at a reduced cost and time.

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# Abstract (in Korean)

# 그래핀 나노시트를 이용한 타겟 검출

**손유나, 약학과 물리약학전공, 약학대학, 서울대학교**

이 논문은 reduced graphene oxide (rGO)를 이용한 광학 기반 바이오센서의 제조 및 특이적 타겟 검출 방법을 소개하고 있다. 형광 인자가 부착 된 이중나선 앱타머를 rGO에 부착한 후, 타겟 물질에 노출되었을 때의 형광 세기의 변화로 검출을 확인하는 바이오센서이다. 본 연구에서는 검출 물질로 인터페론 감마를 선정하였고 그와 특이적으로 결합하는 인터페론 감마 앱타머를 이용하였다. 이중나선 앱타머의 한 쪽 끝에는 rGO 부착 물질을 결합시키고 다른 쪽 끝에는 형광 인자를 결합시킨 상태로 rGO와 반응시켜주면, rGO의 표면에 앱타머들이 부착된 형태의 바이오센서가 제조된다. 사용된 앱타머의 길이는 36 bp로 rGO의 형광 소거 효과 범위를 벗어나게 된다. 그러

므로 검출 물질 반응 전 바이오센서는 형광을 발하는 상태이다. 하지만 검출 물질, 본 연구에서의 인터페론 감마를 바이오센서에 노출시키게 되면, 앱타머 가닥이 이의 상보적인 핵산서열보다 강한 친화력으로 타겟 물질과 반응하면서 단일나선구조 형태로 타겟 물질과 복합체를 형성하게 된다. 이렇게 형성된 복합체는 rGO의 형광 소거 효과 범위로 들어오게 되어 결국 형광을 소실하게 된다. 이와 같은 형광 소실을 기반으로 하여 타겟 물질을 정량적으로 검출할 수 있는 광학 기반 바이오센서이다. 본 논문에서는 이 바이오센서를 이용하여 PBS와 세럼에서의 인터페론 감마를 100 pg/ml부터 10 ug/ml까지 성공적으로 측정 가능함을 확인하였다. 또한, 다른 사이토카인과는 반응성을 보이지 않으며 타겟 물질인 인터페론 감마만 특이적으로 검출함도 확인하였다. 마지막으로 실질적 가용성 평가를 위해 인터페론 감마 농도가 높아지는 것으로 알려져 있는 HIV 초기 감염 환자의 세럼에서의 인터페론 감마 농도 측정도 짧은 시간 내에 가능함을 확인하였다. 이처럼 본 연구에서는 기존의 검출 방법들에 비해 제조공정 및 검출 방법이 간편하고, 정량적 측정이 수분 내에 완료되며, 저농도의 타겟 물질도 뛰어난 감도로 용이하게 검

출할 수 있는 새로운 방식의 바이오센서를 소개하고 있다.

**Key words:** Nano detectors, Graphene nanosheets, Aptamers, Rapid detection, Target-specificity, Target-sensitivity

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