# Combination of magnetic and non-magnetic graphene oxide to develop chemiluminescent aptasensor capable of rapidly quantifying tumor markers

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## ABSTRACT

Rapid and sensitive chemiluminescent aptasensor capable of rapidly sensing tumor markers in human serum with the combination of magnetic and non-magnetic graphene oxide. Human serum (150 µl) containing tumor markers (e.g., AFP and PSA) was mixed in PBS (pH 7.4, 10 µl) containing magnetic graphene oxide (MGO) and incubated for 30 minutes to remove various biomolecules acting as interferences of chemiluminescent aptasensor. Then, ssDNA aptamer-conjugated Cy 3 (100 µl), capable of capturing a specific tumor marker, in Tris-HCl buffer (pH 7.5) was added in the solution and incubated for 5 minutes. After the incubation, non-magnetic graphene oxide in Tris-HCl buffer (pH 7.5, 100 µl) was added in the solution to capture free ssDNA aptamers based on the principle of pi-pi stacking interaction. Finally, chemiluminescent aptasensor rapidly quantified tumor markers bound with ssDNA aptamer-conjugated Cy3 when 1,1'-oxalyldiimidazole (ODI) chemiluminescent reagents (e.g., ODI, $H_2O_2$). The correlation between highly accurate and sensitive chemiluminescent aptasensor and conventional enzyme immunoassay was good. Based on the research results, it is expected that chemiluminescent aptasensors can be developed to early diagnose and prognose various human diseases.

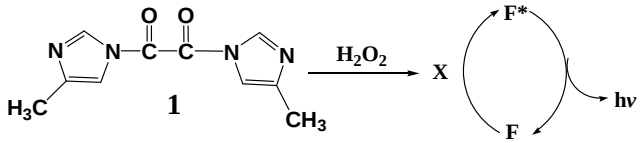
***Keywords***: chemiluminescent aptasensor, magnetic graphene oxide, aptamer, tumor marker

## Introduction

Numerous research groups and companies have developed and commercialized various devices capable of early diagnosing and monitoring human diseases. Unfortunately, most of the devices developed to diagnose critical diseases such as cancer are too complicated and expensive to operate without scientific experience and information.

One major critical disease of males living in highly industrialized country is prostate cancer. Fortunately, it is possible to early diagnose and prognose prostate cancer by quantifying and monitoring prostate specific antigen (PSA) in human serum. Recently, various immunoassays using various detections (e.g., absorbance, chemiluminescence, electrochemicals, fluorescence, mass-spectroscopy) have been developed to detect trace levels of PSA in human serum because the methods can quantify lower concentration of PSA than cut-off value (4 ng/ml in human serum).(*1-5*) However, immunoassays using specific antibodies, capable of binding with PSA, are very complicated, expensive, and time-consuming.

Recently, DNA aptamer capable of capturing PSA in human serum was developed.(*6*) The procedures for synthesizing DNA aptamer are cost-effective, fast, and simple, whereas the production of antibody using small animals (e.g., rat, goat, rabbit) is complicated and very expensive.




**Scheme 1.** ODI-CL reaction. **1**: ODI, **X**: high-energy intermediate formed from ODI-CL reaction, **F**: fluorescent dye under the ground state, **F***: fluorescent dye under the excited state

Scheme 1 shows that the principle of 1,1'-oxalyldiimidazole chemiluminescence (ODI-CL) reaction.(*7-9*) ODI-CL can quantify trace levels of fluorescence materials instead of fluorescence detection. ODI CL detection is more sensitive and selective than absorbance and fluorescence. Fluorescent dye can be labeled with DNA aptamer. It indicates that highly sensitive aptasensor with ODI-CL detection can be developed for the diagnosis of prostate cancer. Thus, I developed for the first time a rapid and simple aptasensor with ODI-CL detection.

## Experimental

### Chemical and materials:

The aptamer (5'-TEX615- TTT TTA ATT AAA GCT CGC CAT CAA ATA GCT TT -3') conjugated with TEX 615 synthesized by Integrated DNA Technologies (Coralville, IA, USA) was used to capture and quantify PSA purchased from Sigma (St. Louis, MO, USA). Bis(2,4,6-trichlorophenyl) oxalate (TCPO) was purchased from TCI-America (Portland, OR, USA). 4-Methylimidazole (4-MImH, 98 %), ammonium hydroxide, $FeCl_2$ and $FeCl_3$ were purchased from Alfa Aesar (Ward

Hill, MA, USA). Hydrogen peroxide ($H_2O_2$, 30 %) were purchased from Mallinckrodt Chemicals (St. Louis, MO, USA). Rhodamine HPLC grade isopropyl alcohol was purchased from Honeywell (Morristown, NJ, USA). Water (HPLC grade), PBS buffer solution (pH 7.4), and ethyl acetate (HPLC grade) were purchased from EMD (Billerica, MA, USA). Graphene oxide was purchased from Graphene Supermarket (Fremont, CA, USA). PBS buffer solution (pH 7.4) was purchased from. Four different Tris-HCl buffer solutions (pH 7.0, 7.5, 8.0, and 8.5) were purchased from Teknova (Holliser, CA, USA).

## Method

### Production of magnetic graphene oxide

0.01 M $FeCl_2$ (100 µl) and 0.01 M $FeCl_3$ (400 µl) was mixed in a 1.5-ml centrifuge tube. Then, 1mg/ ml graphene oxide (400 µl) was added in the centrifuge tube. The tube was in a preheated (85 °C) shaker (Effendorf, Inc). Then, ammonium hydroxide (20 µl) was added in the centrifuge. Finally, chemicals in the tube were reacted to produce magnetic graphene oxide for 50 minutes at 85 °C in the shaker. The stock was stored in a refrigerator.

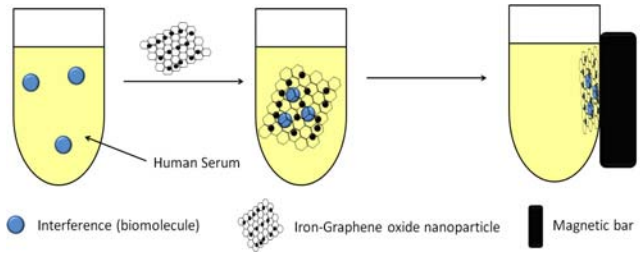



**Fig. 1** Separation of interferences in human serum to develop a highly sensitive chemiluminescent aptasensor

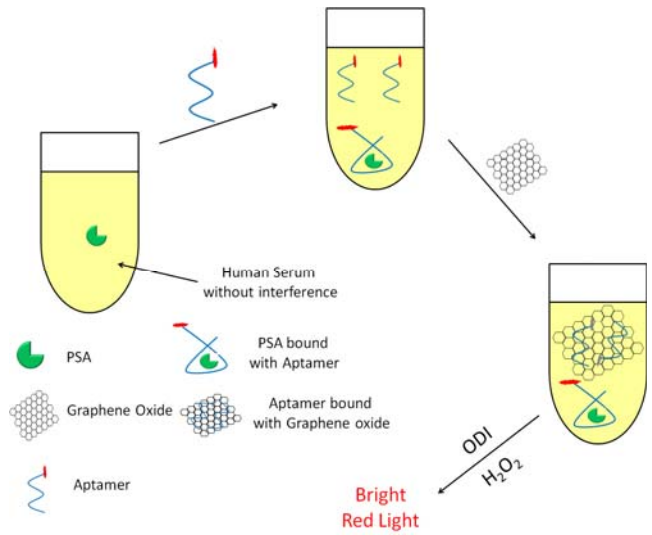



**Fig. 2** Quantification of PSA using chemiluminescent aptasensor

### Procedures

Human serum (200 µl) and 5-time diluted magnetic graphene oxide (200 µl) were mixed in a 1.5-ml centrifuge tube. Then, the mixture was incubated for 30 minutes to remove interferences (biomolecules) in human serum. After the incubation, magnetic graphene oxides containing interferences were separated using a strong magnetic bar as shown in Fig. 1.

Fig. 2 shows the procedure to quantify PSA using chemiluminescent aptasensor. Interference free human serum (100 µl) and aptamer-conjugated TEX 615 (100 µl) were mixed in a 1.5-ml centrifuge tube. The mixture was incubated at 37 °C for 5 minutes. After the incubation, graphene oxide (200 µl) was added in the centrifuge tube. After mixing the solution in the centrifuge tube using a vortex for 1 second, 10 µl of solution was transferred into a borosilicate test tube (12 × 75 mm, VWR). Then, CL emitted with the addition of $H_2O_2$ and ODI in the test tube was measured with Lumat 9507.

### Data analysis

Experimental results obtained in this research were analyzed with appropriate software such as Microsoft Excel.

## Results and Discussion

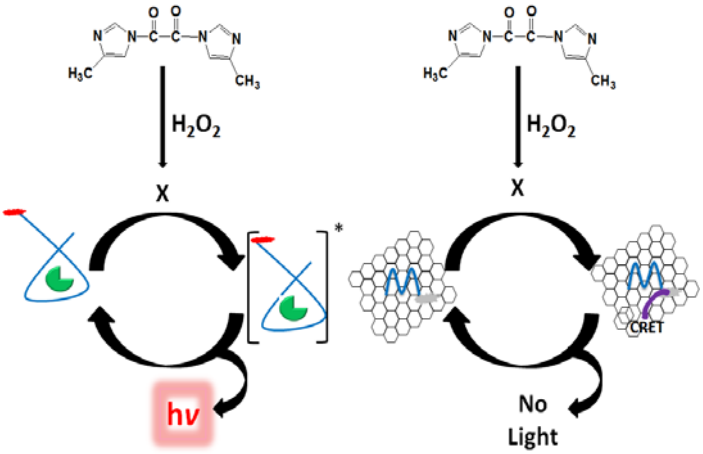



**Fig. 3** Principle of chemiluminescent aptasensor

### Quantification of PSA in PBS buffer

Fig. 3 shows the principle of chemiluminescent aptasensor. As shown in Fig. 3, aptamer-conjugated TEX 615 can capture PSA in solution. And remaining aptamers bind with graphene oxide based on the principle of π-π stacking interaction between aptamer and graphene oxide. With the addition of ODI-CL reagents in the solution, aptamer-conjugated TEX 615 bound with PSA emits red light, whereas aptamer-conjugated TEX 615 bound with graphene oxide can't emit due to the chemiluminescent resonance energy transfer (CRET) from aptamer to

graphene oxide.

Before using human serum, PSA purchased from Sigma was dissolved in Tris-HCl buffer (pH 7.5). As shown in Fig. 4, Chemiluminescent aptasensor can quantify trace levels of PSA from 0.5 to 50 ng/ml. The result indicates that rapid chemiluminescent aptasensor with ODI-CL detection can be developed for the early diagnosis of prostate cancer.

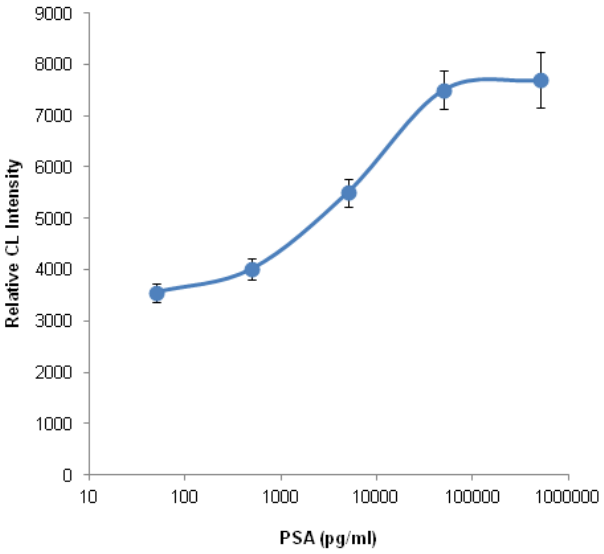



**Fig. 4** Calibration curve to quantify PSA in Tris-HCl buffer

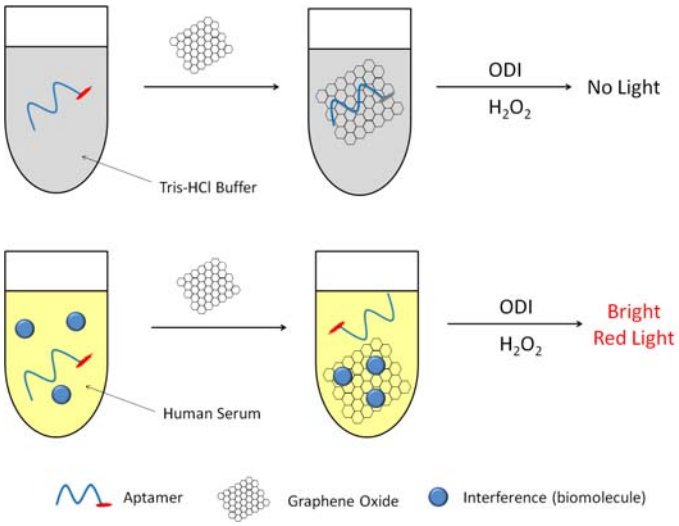



**Fig. 5** Interaction between aptamer and graphne oxide in Tris-HCl buffer and human serum

**Table 1** Comparison of relative CL intensity of human serum with/without interferences

| sample | Relative CL Intensity |
|---|---|
| Human serum with interferences | 278085 |
| Human serum without interferences | 5539 |

**Problem of chemiluminescent aptasensor for quantifying PSA and its solution**

Based on the results shown in Fig. 4, chemiluminescent aptasensor was applied to quantify PSA in human serum. Unfortunately, chemiluminescent aptasensor can't quantify PSA in human serum because graphene oxides bind with biomolecules such as several enzymes instead of aptamer-conjugated TEX 615 as shown in Fig. 5. In order to solve the problem, I synthesized magnetic graphene oxide nanoparticles because they can separate interferences in human serum as shown in Fig. 1. Table 1 shows the effect of magnetic graphene oxide. When graphene oxide was added in human serum not containing interferences, aptamer-conjugated TEX 615 bound with graphene oxide. Thus, the human serum didn't emit light when ODI-CL reagents were added as shown in Table 1. The relative CL intensity measured with human serum not containing interferences was similar to the background noise measured with human serum containing only graphene oxide. However, the human serum containing interferences emitted bright light as shown in Fig. 5. Based on the results, I expect that chemiluminescent aptasensor with ODI-CL detection can quantify PSA for the diagnosis of prostate cancer.

**Quantification of PSA in human serum**

In order to quantify of PSA in human serum, interferences in human serum were removed using the magnetic graphene oxide based on the method shown in Fig. 1. Then, PSA in human serum without interferences were quantified through the same analytical procedures used to quantify PSA in Tris-HCl buffer. The range of linear calibration curve of chemiluminescent aptasensor with ODI-CL detection operated in human serum was similar to that generated in Tris-HCl shown in Fig. 4. In other words, a linear calibration curve capable of quantifying PSA (0.5 ~ 50 ng/ml) in human serum was obtained with the statistically acceptable error range (< 5 %).

## Conclusions

A highly sensitive and rapid chemiluminescent aptasensor with ODI-CL detection was developed for the diagnosis and prognosis of prostate cancer. Also, a method for separating biomolecues such as enzymes and some proteins was developed using magnetic graphene oxide I synthesized. Based on the research results, I expect that

cost-effective aptasensor with ODI-CL detection capable of early diagnosing various diseases and monitoring environmental toxic materials will be developed. Thus, the aptasensor can be applied in various research fields such as biochemistry, biology, clinical chemistry, environmental science & engineering, homeland security, molecular biology, pathology, and toxicology.

Currently, the accuracy and reproducibility of the chemiluminescent aptasensor are being studied. Also, correlation between the new technology and conventional method (e.g., enzyme immunoassay with ODI-CL detection) will soon be reported.

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