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A Bioluminescent Protein-Graphene Oxide Donor-Quencher Pair in DNA Hybridization Assays

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Despite fluorescent quenching with graphene oxide (GO) having shown great success in various applications - bioluminescent quenching has not yet been demonstrated using GO as a quencher. To explore the ability of GO to quench bioluminescence, we used *Gaussia* luciferase (Gluc) as a donor and GO as a quencher and demonstrated its application in sensing of two target analytes, HIV-1 DNA and IFN-γ. We demonstrated that the incubation of Gluc conjugated HIV-1 and IFN-γ oligonucleotide probes with GO provided for monitoring of probe-target interactions based on bioluminescence measurement in a solution phase sensing system. The limits of detection obtained for IFN-γ and HIV-1 DNA detection were 17 nM and 7.59 nM, respectively. Both sensing systems showed selectivity toward the target analyte. The detection of IFN-γ in saliva matrix was demonstrated. The use of GO as a quencher provides for high sensitivity while maintaining the selectivity of designed probes to their respective targets. The use of GO as a quencher provides for an easy assay design and low cost, environmentally friendly reporter.

Introduction

Graphene oxide (GO) is a water-soluble derivative of graphene. Similarly to graphene, GO is comprised of single-layer carbon sheets[1] However, unlike graphene, GO contains several oxygen containing functional groups which allows the GO to disperse itself within an aqueous solution.[2] The availability of these functional groups on the surface makes GO more appealing for biological applications due to its ability to interact with both inorganic and organic materials. GO is capable of adsorption of single stranded DNA (ssDNA) noncovalently via π-stacking interactions and hydrogen bonding.[3] Double stranded DNA (dsDNA) does not adsorb as well as ssDNA on GO.[4] Given all of these characteristics, GO has been exploited in a variety of applications in biosensors.[5] Most notably, GO has been used in fluorescence-based systems[6,7,8] where GO has been shown to be a suitable quencher for fluorophore signals. GO as a biomolecular quencher has been well known and characterized.[9,10,11] Despite this exploitation of GO in predominately fluorescence quenching, bioluminescent quenching using GO is as a quencher has not been reported. Furthermore, the use of this bioluminescence-GO-based quenching technique for nucleic acid has not yet been explored. Based on the success with fluorescent quenching in nucleic acid probes specifically, the use of GO could be an exciting approach to bioluminescent quenching in the development of a variety of bioluminescence-based assays and biosensors. Additionally, GO is considered to be an environmentally friendly and low-cost material. Furthermore, there is no need for a conjugation step to incorporate GO into nucleic acid assays since it binds nucleic acids noncovalently making the assay development simpler. Considering these established and known characteristics of GO as a quencher, we have employed GO as a bioluminescent quencher in DNA hybridization and molecular aptamer beacon (MAB) assays that employ a bioluminescent protein donor.

Bioluminescent proteins are advantageous as reporters due to their low toxicity, wide dynamic range, and higher sensitivity.[12] Additionally, bioluminescence-based reporters does not suffer from high background signals and photobleaching phenomenon that is commonly observed with the use of fluorescent reporters. Several bioluminescent proteins have been isolated, characterized, and employed as optical reporters.[13] One such protein, *Gaussia* luciferase (Gluc) is isolated from a marine organism, *Gaussia princeps*, that is capable of producing a blue light. This light is formed by a reaction between Gluc and its substrate coelenterazine. In the presence of molecular oxygen, the luciferase oxidizes this substrate which results in the release of CO2 and emission of the light at 475 nm. Additionally, Gluc notably is one of the smallest known luciferases at only 19 kDa[14] and exhibits high luciferase activity.[15] Furthermore, Gluc does not require additional co-factors such as Ca2+ or ATP, which simplifies the bioluminescent reaction. This particular luciferase is also durable and able to resist changes in pH or temperature.[16] All

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of these characteristics combined indicate Gluc as being suitable for use as an optical reporter. Our lab has previously demonstrated the use of Gluc as a reporter in bioluminescent hybridization assays for a variety of targets including HIV-1 and interferon gamma (IFN-γ).[17,18] Therefore, these hybridization assay systems can serve as a model to study the use of GO as a quencher in place of traditional quenchers in a bioluminescence-based hybridization assay.

In this manuscript, we investigated the bioluminescence quenching abilities of GO in two different assay systems, bioluminescent molecular aptamer beacon (MAB) assay and bioluminescent DNA hybridization assay for both a signal "turn-on" and a signal "turn-off" method, respectively. In the bioluminescent MAB assay strategy, an MAB is in a closed conformation resulting in graphene oxide to be in close proximity to the bioluminescent protein, Gluc - quenching its luminescence. But upon binding its respective target, the MAB will open and Gluc will be desorbed from the GO resulting in the generation of luminescent signal upon adding its substrate coelenterazine. Using this strategy we designed an assay for interferon-γ (IFN-γ) using MAB that binds IFN-γ. Graphene oxide has been used previously in the literature in conjunction with fluorophores in molecular beacon (MB) assays as a quencher.[19,20] When single stranded DNA sequences adsorbed on GO bind to their target, they change their conformation and form hairpin or dsDNA conformations. These formed structures of a molecular beacon can be easily desorbed from the surface of the GO, allowing for the ability of the luminescence emission to be restored producing a type of "turn on" assay method.[21] Since MAB assay design uses the same principle of MB design we expect the MAB system to work similarly.

In the second assay system we designed a signal turn-off bioluminescence hybridization assay for HIV-1 DNA target. In this strategy, when the target DNA binds the Gluc-labeled probe it forms double stranded DNA (dsDNA) and does not adsorb on GO, therefore the luminescence of Gluc is not quenched. However, if there is no target, the single stranded Gluc-labeled probe can bind to GO quenching the bioluminescence of Gluc. This allows for a dose-dependent change in luminescence in the presence of target. HIV-1 nucleic acid target was employed as an example to demonstrate DNA hybridization strategy using Gluc-GO as donor-quencher pair. A schematic overview of our assay design can be seen in Figure 1. This strategy of utilizing graphene oxide as quencher allows for evaluation of another type of quenchers besides the traditional dye-based quenchers in bioluminescent assays that has not been done previously and may yield better sensitivity and ease of assay development. This method of quenching is favorable as it does not require an additional conjugation step to be incorporated into the assay design.

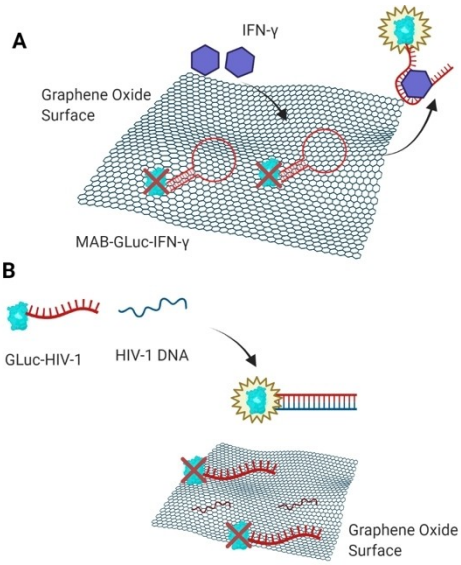

Figure 1. A brief overview of the two assay design strategies with Gluc as a donor and graphene oxide as a quencher. A.) IFN-γ-binding MAB, Gluc-MAB-IFN-γ is incubated with graphene oxide to allow for quenching to occur. Upon addition of IFN-γ there is a conformational change that allows the Gluc-MAB-IFN-γ to separate from the GO and signal is restored. B.) Scheme of the working principle for the DNA hybridization assay for HIV-1 DNA using Gluc as a donor and GO as a quencher. The Gluc-oligonucleotide probe when incubated with the target forms dsDNA. Upon graphene oxide addition only the single stranded Gluc-probe binds to GO resulting in quenching of the bioluminescence signal. Figure made in BioRender.com.

Results and Discussion

Optimization of graphene oxide concentration

Since graphene oxide has not been previously demonstrated as a bioluminescent quencher, the concentration of GO was optimized for the most optimal quenching of the signal. Previous reports with fluorescent experiments involving single stranded DNA (ssDNA) and GO reported the optimal concentration of GO as a quencher to be between 0.25 and 1.5 μg/mL.[22] Therefore, we decided to test GO concentrations within this range and using a constant concentration of Gluc-MAB-IFN-γ (33 nM). This concentration of Gluc-MAB-IFN-γ was determined after optimization assessments (data not shown). Figure 2 A demonstrates the light intensity obtained against varying concentrations of GO. From this data the concentration of 0.3 μg/mL of the GO resulted in a highest signal to noise ratio. As a result for Gluc-MAB-IFN-γ study we decided to use a concentration of 0.3 μg/mL of GO for all further experiments. Additionally, we assessed quenching using similar concentrations of GO and 30 nM probe concentration of Gluc-HIV-1. 30 nM was chosen as the optimal concentration of Gluc-HIV-1 after optimization assessments. An optimal signal to noise ratio was observed at a concentration of 1 μg/ml of GO in this case (Figure 2B). These results indicate that GO can quench bioluminescence signal at 0.3 μg/mL and 1μg/mL with Gluc-MAB-IFN-γ and Gluc-HIV-1 probes, respectively.

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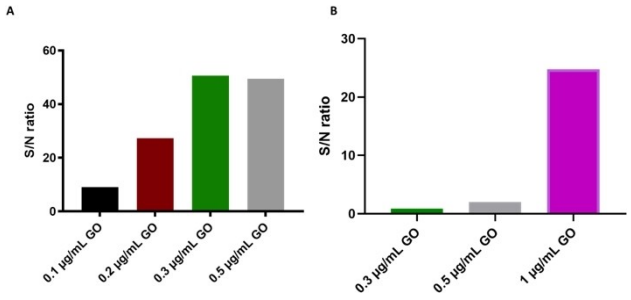

Figure 2. Optimization of Graphene Oxide concentration. A: Signal to noise ratio at varying concentrations of GO when added to the Gluc-MAB-IFN-γ. B: Signal to noise ratio at varying concentrations of GO using a constant concentration of Gluc-HIV-1. The data points are an average of three measurements ±1 standard error of the mean.

Detection of IFN- γ

For the first proof of concept study we aimed to detect IFN-γ utilizing the molecular aptamer beacon using Gluc as a donor and GO as a quencher.[18] This aptamer was chosen as it was already optimized for the selective binding of IFN- γ in our prior studies.[18] We incubated the Gluc-MAB-IFN-γ-GO with a range of concentrations of IFN-γ ranging from 1 nM to 1 μM to generate a dose-response curve which can be seen in Figure 3. Using our system, we can detect IFN-γ as low as 17 nM. The limit of detection was interpolated using a method from Wan et al.[23] This detection limit is within range of other aptamer-based assays and similar to the limit of detection obtained previously by our lab utilizing MAB for IFN-γ.[18,24]

Selectivity of Gluc-MAB-IFN-γ-GO

While aptamers can be designed to recognize different molecules, we wanted to ensure that this molecular aptamer beacon was able to only bind to and detect its target rather than another molecule. Additionally, we wanted to ensure that the graphene oxide itself did not interfere with the selectivity or cause any non-specific signal. In order to assess this, we used increasing concentrations of tumor necrosis factor alpha (TNF-α), bovine serum albumin (BSA), and IFN-γ to incubate with a constant concentration of Gluc-MAB-IFN-γ-GO. These molecules were chosen since TNFα is an immune molecule and BSA is a good, widely accepted control protein. Together these molecules are ideal choices to survey the selectivity of the assay. This assay was performed under the same assay conditions as the dose-response curve experiments. The data in Figure 4 indicates that the system only responded and provided a signal in the presence of IFN-γ. This means that the GO does not interfere with the selectivity of the assay, allowing for both high sensitivity and selectivity.

Detection of HIV-1 nucleic acid

As another example of the utilization of GO as a quencher with Gluc as a donor, we aimed to detect an HIV-1 DNA target utilizing a linear DNA probe sequence complementary to the target DNA. This sequence was chosen as it was previously optimized and utilized by our laboratory in the detection of the HIV-1 target sequence using Gluc as a donor and a traditional quencher molecule (DABCYL).[17] First, our prepared conjugate, Gluc-HIV-1 was incubated for one hour at 37 °C with concentrations of HIV-1 target DNA ranging from 2.5 nM to 100 nM followed by the addition of GO to generate a dose-response curve as depicted in Figure 5. Unhybridized Gluc-HIV-1 probes would bind to the GO in this case as they are single stranded, thereby quenching the Gluc luminescence. We chose to perform target-probe hybridization before adding GO to maximize hybridization opportunity.[25] The data indicates that GO is able to inhibit bioluminescence in this system in the absence of target, while maintaining signal in the presence of a target. The detection limit that we were able to obtain is 7.59 nM. The limit of detection was interpolated using a method from Wan et al.[23] This limit is comparable to and in some cases better than other fluorescence-based DNA hybridization strategies that utilize graphene oxide as a quenching method.[26]

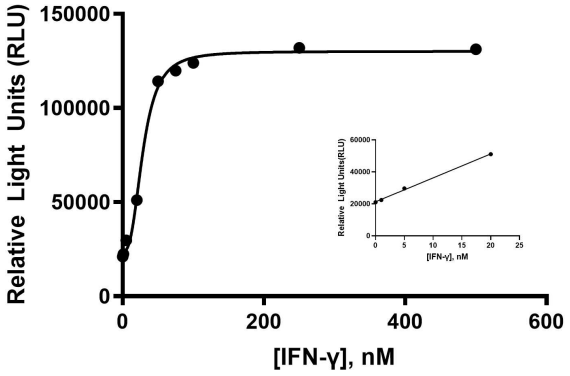

Figure 3. Dose-response curve of IFN- γ. The data points are an average of three measurements ±1 standard error of the mean. The inset graph depicts the area of the curve in the detection limit range for improved visibility. The error bars are obscured by the symbols of the points. Relative light units (RLU) are arbitrary units.

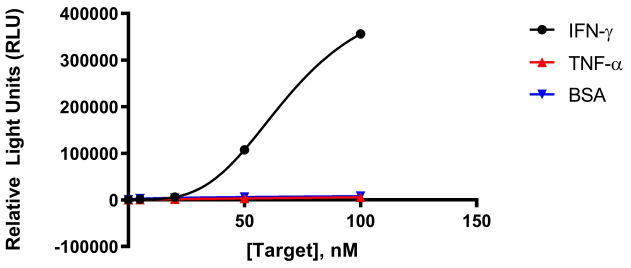

Figure 4. Selectivity assay using increasing concentrations of TNF-α, BSA, and IFN-γ incubated with the Gluc-MAB-IFN-γ-GO complex. The data points are an average of three measurements ±1 standard error of the mean. Some of the error bars are obstructed by the symbols used. Relative light units (RLU) are arbitrary units.

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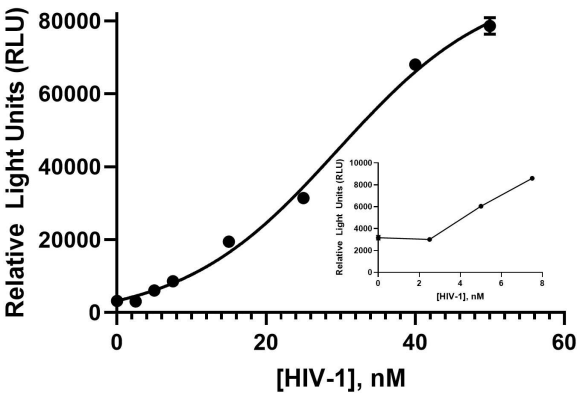

Figure 5. Dose Response curve for HIV-1 target DNA utilizing different concentrations of target with 30 nM HIV-1- Gluc DNA probe and 1 μg/mL GO. Data points are an average of three measurements ±1 standard error of the mean. The inset graph depicts the curve range in the detection limit range for better visualization. Some error bars are obstructed by the symbols of the points of the graph. Relative light units (RLU) are arbitrary units.

Selectivity of Gluc-HIV-1

Selectivity is an important parameter in the development of detection strategies. To assess the selectivity of Gluc-HIV-1 to bind the target HIV-1 sequence and not others, we utilized DNA target with either one (HIVM1) or two mismatches (HIVM2) (Table 1). 25 nM of HIV-1, HIVM1, or HIVM2 DNA was incubated with Gluc-HIV-1 using the same assay conditions as previously described. The data in Figure 6 showed decreased signal was obtained with either the single or double mismatch targets. This indicates the selectivity of our assay to detect only the target HIV-1 DNA and not similar sequences. This selectivity also speaks to the mechanism of action of our assay - as the increased signal with the target indicates specific desorption.

Analysis for matrix effect

To evaluate if the bioluminescence-GO based system would suffer from any real sample matrix issue if sample analysis is performed using this type of system, we generated a dose-response curve in a 1:100 diluted saliva matrix and performed a percent recovery study. The dose-response curve generated in saliva matrix showed similar performance as in the buffer matrix (Figure 7). The detection limit obtained was 1.49 nM and the dynamic range was similar to that obtained with the buffer matrix. A recovery study was performed using known spiked amount of IFN-γ in saliva matrix. We obtained a 104% recovery, which is within the acceptable range. The use of the assay system in serum matrix, however, did not yield acceptable percent recovery, which could pose an issue in sample analysis. However, this could be also related to protein nature of our target analyte, which could bind to serum proteins. Regardless, this data lends itself to an indication of the potential for real-world applications. GO itself does continue to have problems with translatability at the real-world scale however, on account of the adsorption of serum proteins on GO, among others. Future work will be required to overcome some of these translation challenges however this work does lend itself to simplicity of use that would make a real-world application easier.

Conclusion

We have been able to successfully demonstrate, to the best of our knowledge, the first application of graphene oxide as a quencher for a bioluminescent reporter for nucleic-acid probes. We achieved this by utilizing Gluc as the donor and GO as quencher for the detection of IFN- γ and HIV-1 DNA. Each assay

Table 1. Nucleic Acid Sequences used in the study.

Gluc-MAB-IFN-γ	5'-GGG GTT GGT TGT GGG TGT TGT GTC AAC CCC -Azide/-3'
Gluc-HIV-1	5′ - Amine-C6- CTCCGCTTCTTCCTTGTTATGT - 3′
HIV Target DNA	5′ -AATATCAAGCAGGACATAACAAGGAAGAAGCGGAGACAGCGACGAA- 3′
HIV Target 1 Mismatch	5′ - AATATCAAGCAGGACATAACAATGAAGAAGCGGAGACAGCGACGAA - 3′
HIV Target 2 Mismatch	5′ -AATATCAAGCAGGACATAACAATGAAGAAACGGAGACAGCGACGAA - 3′

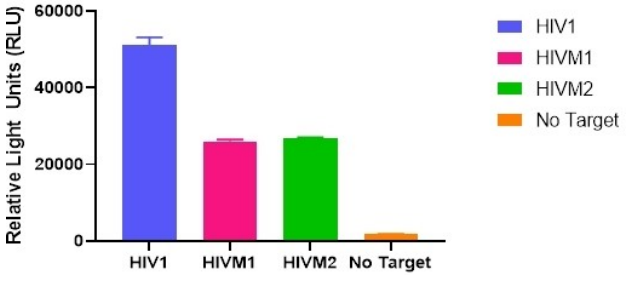

Figure 6. Selectivity of Gluc-HIV-1 using 25 nM of the target sequence, single mismatched sequence, or double mismatched sequence. Sequences are highlighted in table 1. Data points are an average of three data points. Relative light units (RLU) are arbitrary units.

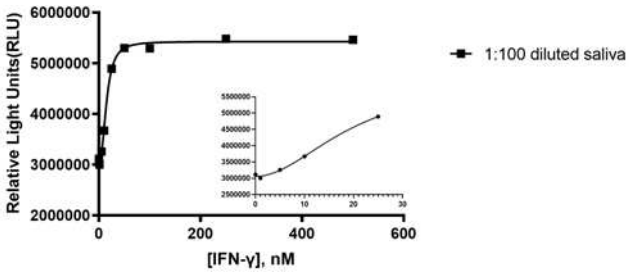

Figure 7. Dose response of Gluc-MAB-IFN-γ-GO in a diluted saliva matrix. Data points are the average of three measurements. Error bars are obscured by the symbols of the graph.

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was able to detect their respective targets sensitively with limits of detection of 17 nM and 7.59 nM for IFN- γ and HIV-1 DNA, respectively. More importantly, the background signals for both systems were low, indicating an efficient bioluminescent quenching by the graphene oxide. The sensitivity of the bioluminescence assay is enhanced using GO as a quencher as compared to DABCYL-based bioluminescent quenching systems. Although, the sensitivity of the assay did not improve significantly the use of GO yields many advantages to the assay. Graphene oxide can quench without the need for any additional conjugation or linking steps - making it a simpler quenching system for bioluminescent proteins. This lack of an additional conjugation step simplifies assays, which could lend itself to enhanced translatability. Additionally, graphene oxide is cost-effective, further enhancing its ability to be introduced into biosensors. We were able to show the use of the assay in real sample analysis although dilution of samples was required to eliminate the matrix effect. Future work will be required to enhance sensitivity and explore the mitigation of existing issues with the translation of graphene oxide systems for use. Based on earlier described characteristics, we believe that graphene oxide has potential to be used as an alternative quenching mechanism for bioluminescent probe-based systems in a variety of future analytical applications. More work needs to be performed with graphene oxide in the future to overcome some of the translatability issues discussed herein, however, this work presents a good starting point for use with molecular beacon type assays using bioluminescence-GO donor quencher pair.

Experimental Section

Expression and purification of *Gaussia* luciferase: *Gaussia* luciferase was expressed using a pCold-1 plasmid vector in E. coli Shuffle strain (New England Biosciences, Ipswich, MA). Cells were grown to an optical density of at least 1.2 in luria broth supplemented with ampicillin. After an incubation of 1 h at 15 °C, cells are induced with a final concentration of 0.3 mM IPTG followed by overnight expression at 15 °C. Cells are then collected by centrifugation at 8,000×g and 4 °C for 20 minutes before being resuspended in 8 mL lysis buffer consisting of phosphate buffer pH 7.6 and 150 mM NaCl. This resuspension is then supplemented with 1x ProBlock™ Gold Bacterial Protease Inhibitor Cocktail (Gold Biotechnology, Inc., St. Louis, MO) and lysed by sonication (Model 500 Sonic Dismembrator, Pittsburgh, PA) for 20 minutes using a one second on/off pulse sequence. This suspension is then centrifuged again at 41,000×g for 30 minutes and the supernatant is filtered twice using a 0.45 μm syringe filter followed by a 0.2 μm syringe filter(VWR, Radnor, PA). The filtered crude product is then incubated with 1 mL Ni-NTA agarose (Qiagen, Valencia, CA) for 10 mL culture in a Pierce™ Centrifuge Column (Life Technologies, Grand Island, NY) by gravity flow, washed with 5 column volumes of lysis buffer followed by a 1 column volume wash buffer containing 50 mM Imidazole. The protein is then eluted with the same buffer containing150 mM imidazole. The elutions are conducted in 1 mL increments. The purified protein is then dialyzed using a Slide-A-Lyzer™ Dialysis Cassette (Life Technologies, Grand Island, NY) with a 10 kDa molecular weight cut-off (MWCO) into phosphate buffer with 150 mM NaCl pH 7.4. Protein concentration is determined using the Bicinchoninic Acid (BCA) Protein Assay Kit (Pierce Biotechnology, Rockford, Illinois) as per manufacturer's protocol. Protein purity is also confirmed using SDS-PAGE analysis.

Conjugation of IFN- γ-binding molecular aptamer beacon and Gluc, Gluc-MAB-IFN-γ: A conjugation step is first performed between Gluc and dibenzocyclooctyne (DBCO). 8 equivalents of DBCO is added to a amber-colored microcentrifuge tube containing one equivalent Gluc and the solution is incubated with shaking for 4 h. Dialysis is then performed using a PBS buffer pH 7.4 consisting of 100 mM sodium phosphate and 150 mM NaCl. Then the molecular aptamer beacon is incubated for 5 min at 80 °C and 1 h at 37 °C with one equivalent of the biotinylated IFN-γ probe (See table 1). 1.5 equivalent of Gluc-DBCO is added and incubated overnight with 1 mL Streptavidin resin (Gold Bioscience, St. Louis, Missouri) per 15–30 μg biotin-aptamer. Unreacted protein is removed using centrifuge columns (Pierce Centrifuge Columns, 0.8 mL, ThermoFisher Scientific, Rockford, IL) by washing with PBS buffer (pH 7.4). Concentration of Gluc-MAB-IFN-γ was then determined by absorbance using SpectraMax 190 Microplate Reader. Samples were concentrated using a vacuum centrifugation (Savant SPD101B SpeedVac, ThermoFisher Scientific, Waltham, MA). Conjugation was confirmed with SDS-PAGE stained with a Silver Stain Kit (ThermoFisher Scientific, Rockford, IL) as per manufacturer's instructions.

Conjugation of HIV-1 probe and Gluc: Gluc is expressed as described above and verified with SDS-PAGE for molecular weight and purity. Gluc is then conjugated to the MB utilizing the chemistry between succinimidyl 6-hydrazinonicotinate acetone hydrazine (Hynic) and succinimidyl4-formylbenzoate (S-4FB). This is done by adapting a protocol from Solulink Biotechnologies (http://www.solulink.com/). Briefly, the DNA probe with a 5'-amino modification is converted chemically to a benzaldehyde by adding 50x excess of succinimidyl 4-formylbenzoate (SFB) in modification buffer containing 100 mM sodium phosphate buffer pH 7.4 and 150 mM sodium chloride. Simultaneously, Gluc lysine residues are modified with 20x excess of succinimidyl 4-hydrazinonicotinate acetone hydrazone (HyNic). Both reactions are incubated for 2 h at room temperature. Reactions are then desalted into the conjugation buffer (100 mM sodium phosphate, 150 mM sodium chloride, pH 6.0) using Zeba desalt spin columns as per manufacturer protocol. Following this, the SFB-modified oligonucleotides are added in 4x excess to the hydrazine modified Gluc and then allowed to react for 2 h at room temperature followed by a reaction over night at 4 °C. SDS-PAGE analysis confirmed the successful conjugation. Following conjugation, un-conjugated probe is removed using Ni-NTA agarose resin. Conjugation mixture is incubated in 0.5 mL Ni-NTA agarose resin in a Pierce Centrifuge Column for 1 h to allow for the His-tag of Gluc to bind the resin. The flow through is then discarded as it contains the unconjugated probe. The Gluc-HIV-1 probe and unconjugated Gluc are then eluted. To remove unconjugated protein, 20 μM of biotinylated HIV probe is added to 400 μL streptavidin resin in a Pierce centrifuge column. This slurry is allowed to incubate with the elution mixture for at least 2 h at 37 °C to allow for hybridization of the MB to the probe. The flow through is then discarded as it contains unconjugated Gluc. The Gluc-HIV-1 probe is then eluted off the column using hot water dehybridization. The purified conjugation is then verified with SDS-PAGE and silver staining using Thermo Scientific Pierce Silver Stain kit as per the manufacturer's instruction.

Preparation of graphene oxide conjugates and optimization: Graphene oxide is diluted in nuclease free water to a final concentration of 0.5 mg/mL and sonicated with an ultrasonic cleaner (Symphony VWR Ultrasonic Cleaner, Westchester, PA) for 3 h. Prior to experiments, GO is diluted to the desired concentration and added to the Gluc-MAB-IFN-γ or Gluc-HIV-1 and incubated for 30 minutes at room temperature with gentle shaking. For optimiza-

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tion studies, the solution was diluted to a final concentration of either 0.1, 0.2, 0.3, or 0.5 μg/mL and incubated with a constant concentration of Gluc-MAB-IFN-γ to prepare Gluc-MAB-IFN-γ-GO. For Gluc-HIV-1 we used concentrations of 0.3, 0.5 and 1 μg/mL to prepare GlucHIV-1-GO. Light is then measured using the CLARIOstar reader after injection of 100 μL coelenterazine to a final concentration of 0.0025 mg/mL.

Detection assay for IFN-γ: Briefly, 90 μL of Gluc-MAB-IFN-γ-GO complex is added to varying concentrations of IFN-γ from 0 to 500 nM for a total reaction volume of 100 μL. This is then incubated for one hour at room temperature before the addition of 100 μL coelenterazine to a final concentration of 0.0025 mg/mL and quantifying bioluminescence using a CLARIOstar Reader as previously described (BMG Labtech, Cary, NC).

Specificity of Gluc-MAB-IFN-γ-GO: Increasing concentrations from 0 to 100 nM of either IFN-γ, TNF-α, or BSA were incubated with a constant concentration of Gluc-MAB-IFN-γ-GO for 1 h at room temperature. After incubation, 100 μL coelenterazine with a final concentration of 0.0025 mg/mL was injected and bioluminescence was quantified as previously described.

Detection of HIV-1 target DNA: An aliquot of 10 μL of the synthetic DNA target (Table 1, HIV-1 target DNA), in hybridization buffer (10 mM Tris pH 7.4 with 500 mM NaCl, 1 mM MgCl2, and 0.1% Tween-20), at concentrations ranging from 2.5 to 100 nM is added into a 96-well non-binding black microplate. 30 nM of Gluc-HIV-1 is added to each well for a total volume of 100 μL. The reactions are then incubated for 1 h at 37 °C before the addition of 1 μg/mL GO. Reactions are then incubated for 1 h at 37 °C. Bioluminescence signal is then measured after the injection of 100 μL coelenterazine to a final concentration of 0.0025 mg/mL with the CLARIOstar as previously described.

HIV-1 selectivity: Assay Synthetic DNA with either 0, 1, or 2 mismatches is incubated in varying concentrations with 30 nM Gluc-HIV-1-GO in hybridization buffer (10 mM Tris pH 7.4 with 1 mM MgCl2, 500 mM NaCl, and 0.1% Tween-20) in a 96-well non-binding black microplate. Reactions are then incubated for 1 h at 37 °C before the addition of 1 μg/mL GO is added. Reactions are then incubated for 1 h at 37 °C. Signal is then measured as previously described upon injection of 100 μL coelenterazine to a final concentration of 0.0025 mg/mL.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords: assays · bioluminescence · DNA hybridization · graphene oxide · molecular beacons

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