Development of a Split Light-Up Sulforhodamine B Aptamer for Monkeypox Virus Detection

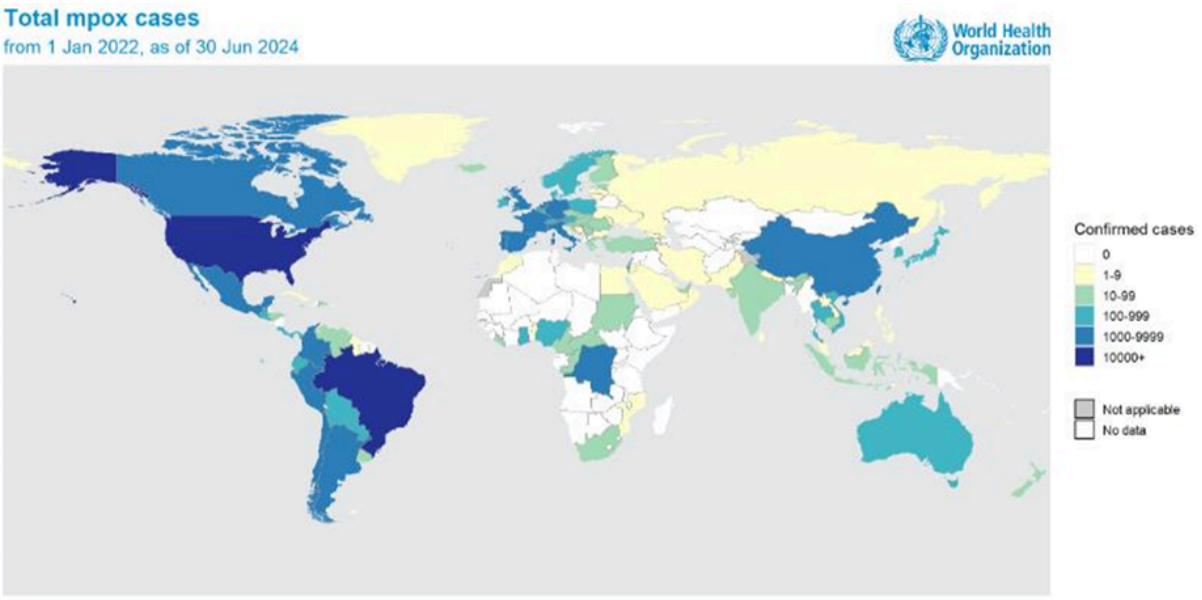
Abstract:

This study developed a fluorogenic aptamer sensor for rapid and accurate detection of monkeypox virus (Mpox)—a challenging infectious disease to diagnose due to clinical similarities with chickenpox and skin infections. By splitting the SRhB aptamer into two optimized oligonucleotide strands and controlling experimental conditions such as pH, NaCl and $MgCl_2$ concentration, and incubation time, this method achieves stable fluorescence signals even at low DNA concentrations. Additionally, targeting three different locations on the viral DNA with multiple sensors increased sensitivity, allowing detection down to 0.5 nM DNA concentration. The results indicate high selectivity of the sensor, effectively distinguishing Mpox DNA from other DNA. This method shows broad potential for rapid diagnosis of infectious diseases, particularly in settings with limited access to complex diagnostic equipment, contributing significantly to disease prevention and control efforts.

Keywords: Aptamer sensor, Monkeypox (Mpox), Fluorescence, Sensitivity and selectivity, Rapid detection.

1. REASONS FOR CHOOSING THE TOPIC

Monkeypox (Mpox) is a zoonosis disease caused by the monkeypox virus. This virus is divided into two main branches: Branch I, which is commonly found in Central Africa and causes severe illness, and Branch II, which mainly appears in West Africa and leads to milder disease [1]. In 2022, branch IIb of this virus triggered a global outbreak, primarily affecting men who have sex with men and individuals in close contact with infected persons [2].



Geographic distribution of confirmed monkeypox cases reported or identified by the World Health Organization (WHO) from publicly available sources, from January 1, 2022, to June 30, 2024. (Source: WHO)

The monkeypox virus was first discovered in 1958 when it appeared in monkeys in a laboratory in Denmark, and the first human case was recorded in Africa in 1970 [1, 3]. Since the 1990s, monkeypox has spread more widely and become more dangerous. By July 2022, the World Health Organization declared a global health emergency due to the disease, with over 90,000 cases reported worldwide by the end of 2022 [4] (Figure 1).

In Vietnam, monkeypox has also emerged. According to reports from the Pasteur Institute of Ho Chi Minh City, the southern provinces of Vietnam have recorded 199 cases, with 8 deaths reported between 2023 and 2024.

Monkeypox is transmitted through direct contact with damaged skin or mucous membranes, as well as through bodily fluids from infected humans or animals [1]. The main symptoms include fever, swollen lymph nodes, and rashes, but these can easily be confused with other diseases like chickenpox or skin infections. The most accurate diagnostic method currently available is real-time polymerase chain reaction (RT-PCR), but this requires complex equipment, high-level technical expertise, and high costs [5]. This makes it difficult to detect and control the disease in regions with limited resources, such as parts of Africa or several provinces in Vietnam. Therefore, developing a new, rapid, and simple method for detecting the monkeypox virus is urgently needed.

Several more affordable alternatives to current diagnostic methods have been explored, with a focus on the use of nanomaterials and aptamers. Aptamers are nucleic acids selected to specifically bind to a variety of target molecules, ranging

from small molecules and toxins to intact cells, and they have broad applications in biosensing methods [6]. With high affinity and a short length, typically under 100 nucleotides, aptamers are easy to synthesize, which reduces costs and increases production potential compared to antibodies. Notably, aptamers can exhibit fluorescence when binding with certain substances, making them highly useful for developing label-free, low-cost biosensors [7]. These aptamers have already been successfully applied in intracellular imaging analysis [8], and their potential applications are still vast. Therefore, **this study focuses on developing a fluorogenic aptamer sensor for the rapid and accurate detection of Mpox, contributing significantly to public health protection.**

2. RESEARCH QUESTIONS AND SCIENTIFIC HYPOTHESIS

2.1. Research Questions

How can an SRhB aptamer variant be developed to detect the Mpox virus with high sensitivity and selectivity?

What reaction conditions (such as pH, NaCl concentration, $MgCl_2$, and incubation time) can optimize the fluorogenic signal of the aptamer sensor in detecting the Mpox virus?

To what extent can the SRhB aptamer sensor differentiate between the DNA of the Mpox virus and the DNA of other pathogens?

How can using multiple targeted sensors directed at different positions on the DNA sequence improve the detection limit and enhance sensitivity in real-world applications?

2.2. Scientific Hypotheses

When combined with Sulforhodamine B (SRhB), the SRhB aptamer will fluoresce upon recognizing and specifically binding to the DNA of the monkeypox virus (Mpox).

The sensitivity and selectivity of the method will be enhanced by optimizing reaction conditions (such as pH, NaCl concentration, $MgCl_2$, and incubation time), allowing for the detection of low DNA concentrations and clear differentiation between Mpox virus DNA and other DNA types.

The use of multiple sensors targeting different regions of the Mpox DNA sequence will improve the detection limit and enhance the fluorescent signal, enabling the sensor to detect the virus at lower concentrations than methods using a single sensor.

This SRhB aptamer sensor method can provide a rapid, accurate, and cost-effective diagnostic solution, particularly for regions with limited access to complex diagnostic equipment. It may also have broad applications in the detection of other infectious diseases.

3.1. Chemicals

Table 1. Chemicals used in the experiment

Chemical Name	Source	Purity
Aptamer	Phusa Biochem LTD	>95%
Sodium biphotphate	Merck, Germany	PA
Sodium chloride	Merck, Germany	PA
Magnesium chloride	Merck, Germany	PA
Sulfor Rhodamine B	Merck, Germany	PA

3.2. Apparatus and Equipment

- Fluorescence Spectrometer F-4700 (Hitachi) with excitation wavelength of 565 nm and emission spectrum range from 565 to 586 nm.
- pH Meter HANNA HI 2211-02.
- Scientech SA210 Analytical Balance with accuracy of 0.0001g.
- Centrifuge for 0.2/1.5/2 ml centrifuge tubes, 4000 rpm.
- Micropipettes: 2-20 µL, 20-200 µL, 100-1000 µL.

3.3. Experimental Procedure

3.3.1. Fluorescence Measurement and Target DNA Detection

Prepare two test tubes for the experiment, where the first test tube contains 25 µL of SM-R solution (10 µM), and the second test tube contains 25 µL of SM-L solution. Both solutions will then be mixed with 500 µL of pH 7.2 buffer, which consists of 50 mM NaCl, 10 mM NaP, and 5 mM $MgCl_2$, to create an optimal environment for the reaction. Next, add 30 µL of 10 µM SRhB solution to each test tube. After completing the preparation steps, the first test tube will not contain target DNA (control sample), while the second test tube will be supplemented with 25 µL of 10 µM target DNA solution to test the interaction between the components in the sample and the target DNA. After mixing, the contents of both test tubes will be centrifuged to ensure uniform mixing of components, and then allowed to stand for 15 minutes to facilitate the reaction. Finally, the fluorescence intensity of the samples will be measured using a fluorescence spectrometer, recording the fluorescence signal at the excitation wavelength of 546 nm and emission wavelength of 578 nm. This will allow the evaluation of changes in fluorescence upon the presence of target DNA in the test samples.

3.3.2. pH Study

To investigate the effect of pH on the reaction process, a series of test tubes were prepared with the following components: each test tube contains 25 μL of SM-R solution (10 μM) and 25 μL of SM-L solution. These solutions were then mixed with 30 μL of 10 μM SRhB solution and 500 μL of buffer consisting of 50 mM NaCl, 10 mM NaP, and 5 mM MgCl2. Specifically, these test tubes were prepared with different pH values of 6.3, 6.6, 6.9, 7.2, 7.5, 8.0, and 8.7 to examine the pH-dependent changes in the reaction. In each test tube, the first test tube does not contain target DNA (used as a control sample), while the second test tube contains 25 μL of 10 μM target DNA solution. After mixing the components, the contents of each test tube are centrifuged to ensure uniform mixing. Then, the test tubes are allowed to stand for 15 minutes for the reaction to complete. This procedure helps assess the effect of different pH levels on the interaction between the components of the reaction, and provides insights into the changes in fluorescence corresponding to each pH value.

3.3.3. Selectivity Study

To investigate the selectivity of the aptamers, a preparation procedure was performed with the following test tubes: each test tube contains 25 μL of SM-R solution (10 μM) and 25 μL of SM-L solution, followed by mixing with 500 μL of pH 7.2 buffer, consisting of 50 mM NaCl, 10 mM NaP, and 5 mM MgCl2, to create a stable reaction environment. Next, 30 μL of 10 μM SRhB solution was added to each test tube. The first test tube does not contain target DNA (used as the control sample), while the subsequent test tubes are added with 25 μL of various target DNA solutions: target DNA (10 μM), target DNA6mut (10 μM), and target DNAC11T (10 μM), to study the interaction of the aptamer with different DNA target variants. After mixing the components, the contents of the test tubes are centrifuged to ensure uniform mixing. The test tubes are then left undisturbed for 15 minutes to allow the reactions to occur. Finally, the fluorescence intensity of the samples is measured using a fluorescence spectrometer, recording the fluorescence signal at an excitation wavelength of 546 nm and an emission wavelength of 578 nm. By measuring the fluorescence, the selectivity of the aptamers for each type of target DNA and its variants can be assessed.

3.3.4. Investigation of the MgCl2 effect

To investigate the effect of salt concentration on the experiment, a series of test tubes were prepared with the following components: each test tube contains 25 μL of SM-R solution (10 μM) and 25 μL of SM-L solution, followed by mixing with 500 μL of pH 7.2 phosphate buffer containing 50 mM NaCl. Next, 30 μL of 10 μM SRhB solution was added to each test tube to enhance fluorescence. The first test tube does not contain target DNA (used as the control sample), while the second test tube contains 25 μL of 10 μM target DNA solution. Then, different concentrations of

MgCl2 were added to each test tube: 25 μL, 50 μL, 75 μL, and 100 μL of 100 mM MgCl2 solution to examine the effect of MgCl2 salt concentration on the reaction. After mixing the components, the contents of the test tubes are centrifuged to ensure uniform mixing of components. The test tubes are left undisturbed for 15 minutes to complete the reactions. Finally, the fluorescence intensity of the samples is measured using a fluorescence spectrometer, recording the fluorescence signal at an excitation wavelength of 546 nm and an emission wavelength of 578 nm. This procedure allows for the evaluation of the effect of MgCl2 salt concentration on the fluorescence efficiency in the experiment.

3.3.5. Identification of Potential Risks and Safety Warnings

During the experiment, the experimenter needs to be aware of certain risks to ensure safety and accuracy in the working environment.

From a biological perspective, some chemicals used in the experiment can be harmful to health if directly exposed. For example, SRhB can cause skin and eye irritation, and inhalation may lead to coughing, throat irritation, or difficulty breathing. To avoid harmful effects from SRhB, the experimenter should wear proper protective equipment such as gloves, safety goggles, and a lab coat, and conduct the experiment in a well-ventilated area to limit exposure to the respiratory system. If SRhB accidentally contacts the body, the affected area should be washed thoroughly with water and monitored. In case of unusual symptoms, medical advice should be sought.

From an environmental standpoint, improper disposal of chemicals used in the experiment can be harmful. For instance, magnesium chloride (MgCl2) when released in large quantities can damage soil, making it difficult for plants to absorb water and nutrients. SRhB can also contaminate water sources if not disposed of properly. To minimize environmental impact, all waste must be collected and disposed of following regulations regarding waste disposal and biosafety.

Regarding equipment-related risks, special care should be taken when working with electrical devices to avoid the risk of electric shock or fire. Additionally, to ensure the accuracy of experimental results, the experimenter should use separate tools for each sample, clean and disinfect the work surfaces and instruments to prevent cross-contamination.

4. RESULTS AND DISCUSSION

4.1. Split SRhB Aptamer Design

The SRhB aptamer is a DNA molecule that has been previously studied for its ability to bind to the dye Sulforhodamine B (SRhB), enhancing the fluorescence signal of SRhB [9] (Figure 2). To utilize this aptamer for detecting the DNA of the monkeypox virus (Mpox), the SRhB aptamer will be split into two DNA

oligonucleotide segments. When these DNA fragments are separate, they do not exhibit fluorescence like the original SRhB aptamer and are referred to as the split SRhB aptamer (Figure 2).

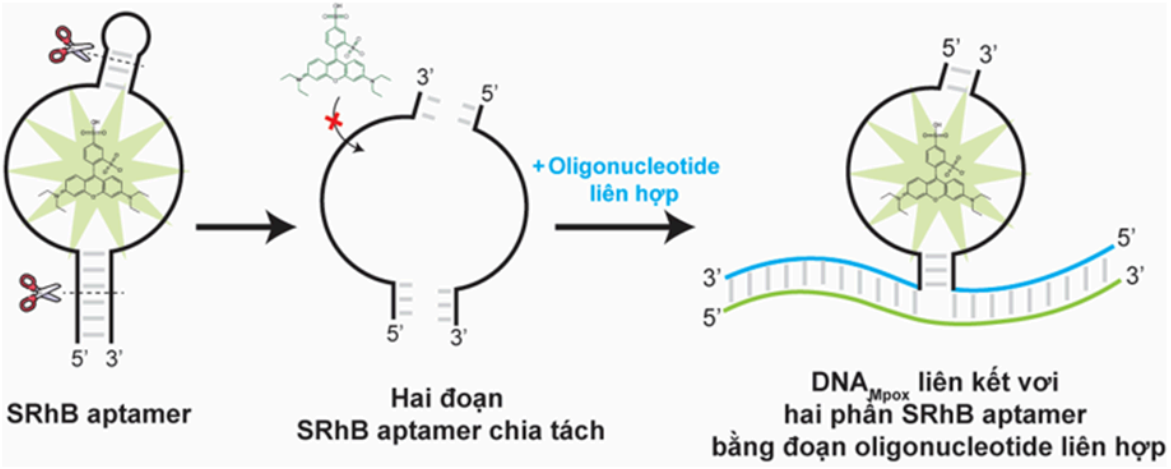


Figure 2. Diagram of the split SRhB aptamer design for detecting Mpox virus DNA. In the diagram, the DNA segment specific to the Mpox virus is represented in orange, while the conjugated oligonucleotide segments, which facilitate recognition and binding to the viral DNA segment, are represented in blue.

Subsequently, these two DNA oligonucleotide fragments will be linked to complementary oligonucleotide segments that can recognize and bind to the target DNA of the Mpox virus. When these oligonucleotide segments connect with the virus DNA, they bring the two split SRhB aptamer segments closer together. This enables the two aptamer segments to reassemble the original structure of the SRhB aptamer and bind to the SRhB dye, enhancing the fluorescence signal (Figure 2).

To split the SRhB aptamer into two strands, the research team must first identify a suitable position within the SRhB aptamer structure to serve as the cleavage point, allowing one strand to be separated from the other. This must be done carefully, as many positions within the aptamer structure are crucial for its folding and functionality (marked in red in Figure 3A). If the aptamer is split at these positions, its functionality may be compromised, preventing the two strands from rejoining. However, there are other splitting positions that allow the SRhB aptamer to be divided while maintaining a strong enough bond between the two strands for the aptamer to continue functioning and fluorescing.

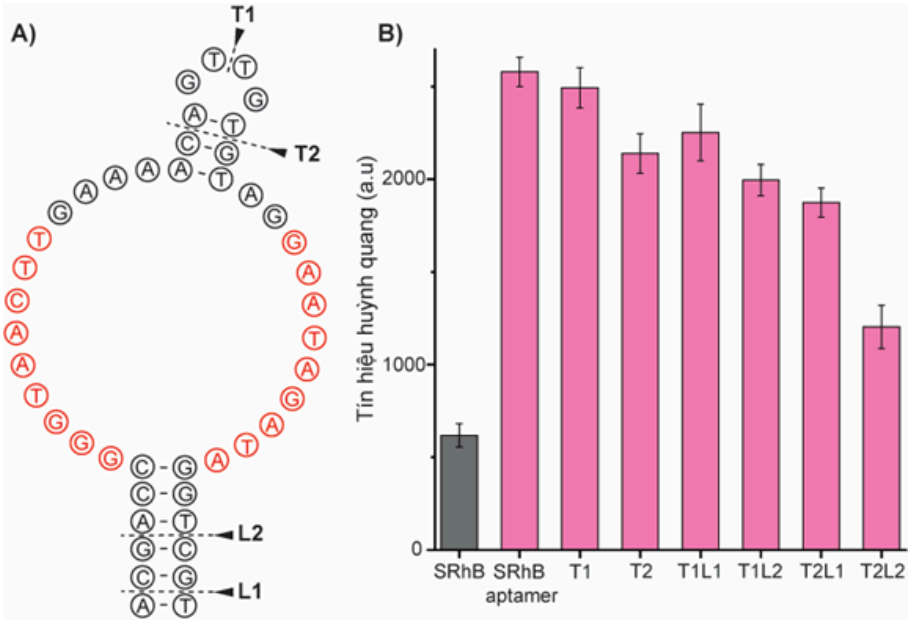


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Figure 3. Study of the cleavage position of the SRhB aptamer. (A) Diagram of the cleavage positions on the SRhB aptamer and (B) Fluorescence signal of the aptamers in the presence of SRhB.

In order for the two strands, after being split, to generate a fluorescence signal upon binding to the target DNA, they must not interact with each other in the absence of DNA. To reduce the background fluorescence signal of the SRhB aptamer strands, the research team will test different cleavage positions (Figure 3A). These positions will be examined to determine the appropriate length of the aptamer strands after splitting, in order to prevent the two strands from reannealing with each other while still retaining the ability to bind to SRhB like the original aptamer. The results show that when the SRhB aptamer is cleaved at positions T2 and L2 (T2L2 sample in Figure 3B), the fluorescence signal is minimized but still significantly higher than the signal from SRhB alone. This indicates that the two strands are still able to combine to form the SRhB aptamer structure and bind to SRhB.

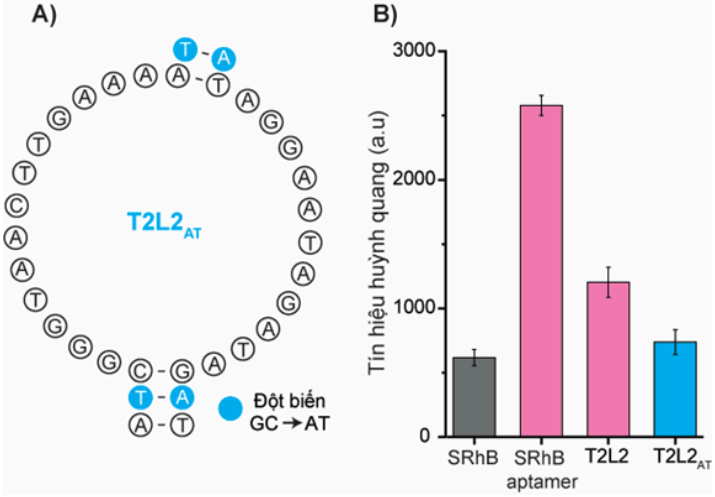


Figure 4. Study of the nucleotide pair changes from G/C to A/T. (A) Positions of the nucleotide changes and (B) Fluorescence signal of the aptamers in the presence of SRhB.

To reduce the interaction between the two strands of the SRhB aptamer after splitting, the research team replaced two G/C base pairs with A/T pairs (T2L2AT, Figure 4A). This is because the G/C pair is held together by three strong hydrogen bonds, while the A/T pair has only two weaker hydrogen bonds, which helps reduce the interaction between the two separated strands. The results show that this change has a significant effect, reducing the fluorescence to a level almost equivalent to the sample containing only SRhB (Figure 4B). Therefore, in addition to adjusting the strand length, altering the number of G/C pairs also helps reduce the interaction between the split strands. The optimized sequence of the two strands will be used in the subsequent steps.

4.2. Reconfiguration of the two SRhB aptamer strands after splitting for Mpox DNA detection.

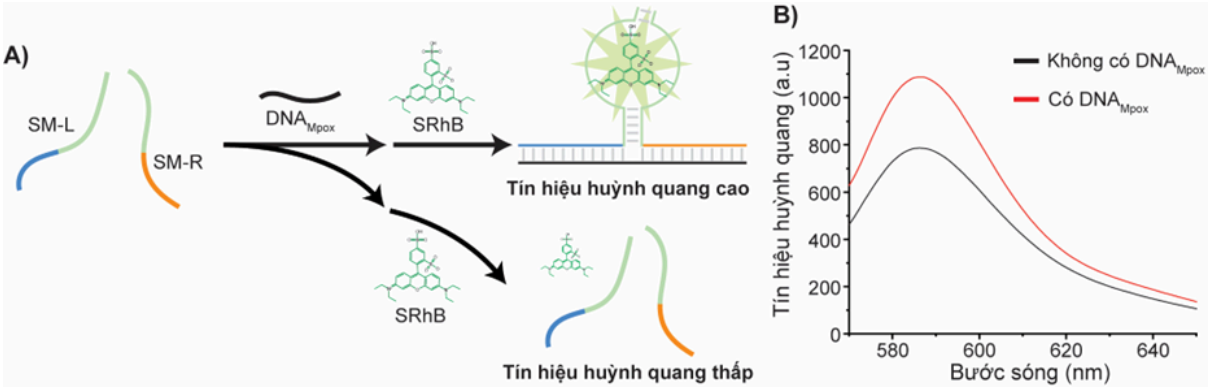


Figure 5.
(A) Diagram of the sensor developed from the two split strands of the SRhB aptamer along with two complementary strands for target DNA.
(B) Fluorescence signal when DNA Mpox is absent and when it is present.

After splitting the SRhB aptamer into two optimal oligonucleotide strands (T2L2AT) in previous experiments, each strand was further studied and adjusted for application in the detection of Mpox virus DNA. After separation, each strand (represented by the green segment in Figure 5A) is supplemented with short oligonucleotide sequences at the ends (denoted by the light blue and red segments in Figure 5A). The nucleotide sequences of these two supplementary segments are designed to correspond to a region of the Mpox virus DNA, allowing them to bind with the target DNA and bring the two strands of the SRhB aptamer closer together. This facilitates the folding and restructuring of the SRhB aptamer core, enabling it to bind with SRhB and emit fluorescence (Figure 5). The two strands of the SRhB

aptamer, after adding the supplementary segments from the target DNA, will be referred to as SM-L and SM-R.

To develop a sensitive and stable method for detecting Mpox virus DNA, the distance between the junctions of the target DNA and the two oligonucleotides of the SRhB aptamer will be optimized (Figure 6A). The sensor signal heavily depends on the three-dimensional structure (three-way junction, 3WJ) formed by SM-L, SM-R, and the target DNA. In natural DNA 3WJ structures, there are usually unpaired nucleotides between the three branches, which can affect the binding of the three DNA strands. Therefore, SM-L and SM-R will be designed with unpaired Thymine (T) nucleotides, which are inserted to connect the supplementary segments with the target DNA and the split SRhB aptamer strand (Figure 6A). These inserted nucleotides, called "linkers," are labeled as T0, T1, and T3, corresponding to 0, 1, or 3 Thymine nucleotides. When these two strands bind with the target DNA, the resulting 3WJ structure will fold differently. Some configurations may generate a more suitable 3WJ structure, helping to restore the SRhB aptamer structure from the two separated strands.

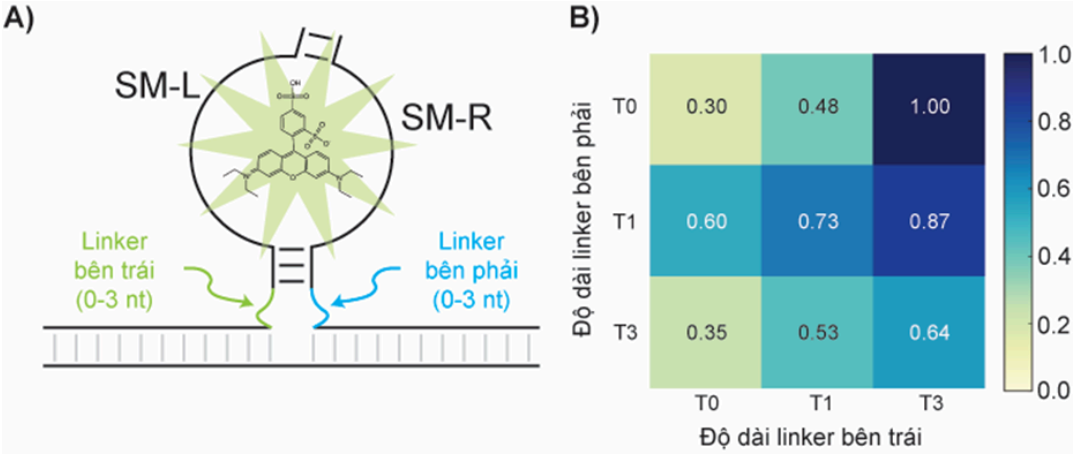


Figure 6. (A) Diagram of the sensor developed from the SRhB aptamer, showing the positions of the two linker segments. (B) Heatmap representing the change in fluorescence signal with and without the presence of target DNA, for each pair of different linker segment lengths.

Different linker length combinations for the left and right linkers were tested under both conditions with and without the presence of target DNA to optimize the structure of SM-L and SM-R (Figure 6). For each combination, the background signal under the condition without target DNA was subtracted, and the fluorescence of each combination was represented as a heatmap (Figure 6B). Some combinations did not show an increase in fluorescence even with the addition of target DNA, indicating that the linker length could affect the interaction between SM-L and SM-R to recreate the original SRhB aptamer structure. The results showed that when the left linker did not contain Thymine, the fluorescence signal was low in the presence of target DNA. In contrast, when the left linker contained 1 or 3 Thymine nucleotides, the

fluorescence signal significantly increased, highlighting the important role of the left linker in supporting the interaction of the two strands, SM-L and SM-R, to form the SRhB aptamer structure. Among the combinations tested, the one with 3 Thymine nucleotides in the left linker and no Thymine in the right linker produced the best signal. Therefore, this combination will be chosen for subsequent experiments.

4.3. Optimization of analytical conditions

4.3.1 Investigation of Buffer Conditions

After optimizing the structure of the two SM-L and SM-R strands for the detection of Mpox virus DNA, the research team will continue to adjust the analytical conditions to achieve the best measurement signal. Since the fluorescence signal of SRhB and the binding ability between the aptamer and SRhB are significantly influenced by the buffer conditions, these factors will be prioritized for adjustment, including: pH, NaCl concentration, and MgCl2 concentration.

Due to the significant effect of pH on the aptamer-SRhB binding ability, the research team examined a narrow pH range from 6.6 to 8.4 (Figure 7A). To compare the pH conditions, the fluorescence signal increase in the presence of target DNA was used as an indicator. The results showed that a pH of 7.2 generated the highest signal compared to the other pH levels, so this pH value will be used in subsequent experiments.

For NaCl concentrations ranging from 0 to 100 mM, the fluorescence signal reached its highest at 50 mM (Figure 7B). Therefore, this NaCl concentration will be applied to develop a highly sensitive sensor for Mpox virus DNA analysis.

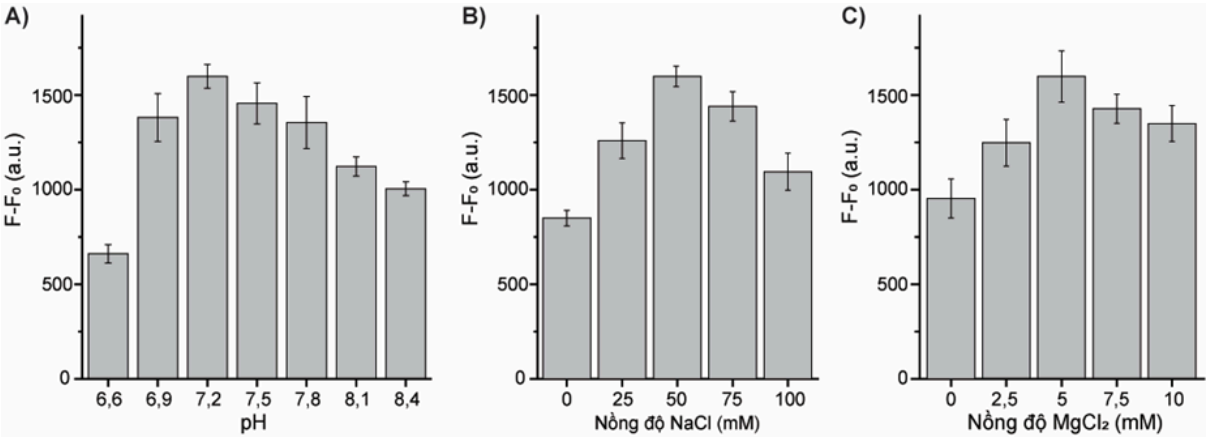


Figure 7. Survey of buffer conditions for Mpox DNA analysis using the two SM-L and SM-R strands.

MgCl2 concentration is also an important factor for the binding ability between the analyte and the aptamer as well as the connection between DNA strands (Figure

7C). Therefore, MgCl2 concentration was surveyed from 0 to 10 mM. The results showed that the fluorescence signal was highest at 5 mM MgCl2 in the presence of target DNA. Hence, this concentration will be used for subsequent research steps.

4.3.2. Investigation of Incubation Time

The incubation time between the SM-L and SM-R strands with the target DNA is crucial to enhance the fluorescence signal and improve the sensitivity of the method. The fluorescence signal was measured every 10 minutes over an 80-minute period after mixing SM-L, SM-R, and target DNA (Figure 8). The results showed that the fluorescence signal remained almost stable after 60 minutes. However, a significant increase in the signal could be observed as early as the first 20 minutes. To achieve a stable and optimal signal, an incubation time of 60 minutes will be chosen for subsequent studies.

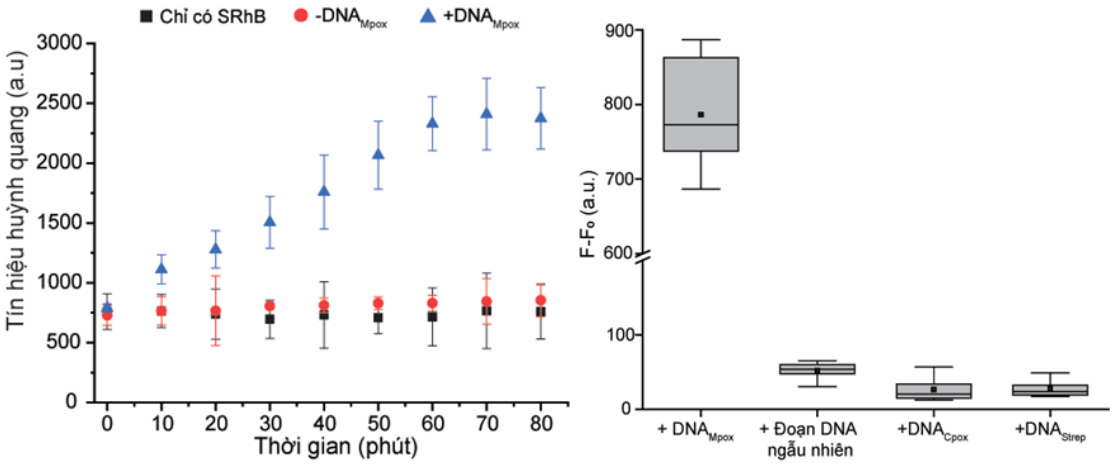


Figure 8. Survey of incubation time between the two SM-L and SM-R strands with Mpox DNA.

Figure 9. Evaluation of the selectivity of the method with different target DNA sequences.

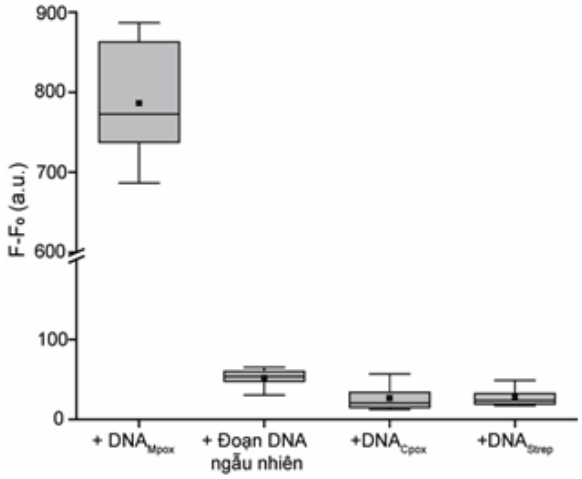
The incubation time between the two SM-L and SM-R segments with the target DNA is very important to enhance the fluorescence signal and improve the sensitivity of the method. The fluorescence signal is measured every 10 minutes over a period of 80 minutes after mixing SM-L, SM-R, and the target DNA (Figure 8). The results show that the fluorescence signal remains almost stable after 60 minutes. However, a significant increase in the signal can be observed as early as the first 20 minutes. To achieve the most stable and optimal signal, a 60-minute incubation time will be chosen for subsequent studies.

After evaluating the conditions, the research team has compiled the following table of optimal parameters for the analysis:

Table 1: Optimal Analytical Conditions

Condition	Parameter
Excitation wavelength	565 nm
Emission wavelength	586 nm
pH	7,2
NaCl	50 mM
$MgCl_2$	5 mM
Incubation time	60 minutes

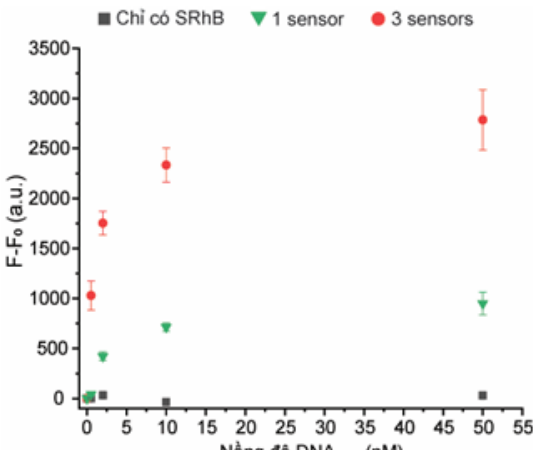
6.4. Evaluation of Method Selectivity



Hình 9: Đánh giá độ chọn lọc của phương pháp với các đoạn DNA đích khác.

The Mpox virus can cause clinical symptoms similar to chickenpox or skin infections, which may lead to misdiagnosis. Accurate diagnosis of the Mpox virus is crucial for effective contact tracing and treatment. Therefore, the analysis method based on the SRhB aptamer will be evaluated for selectivity with different DNA fragments, including: a random DNA fragment, DNA from the chickenpox virus (DNACpox), and DNA from the bacterium *Streptococcus* that causes skin infections (DNAStrep) (Figure 9). The increase in fluorescence signal, both in the presence and absence of target DNAs, will be used to compare selectivity. The results show that in the presence of DNAMpox, the fluorescence signal increases by 8-10 times compared to the other DNAs. This demonstrates that the method developed by the research team has high selectivity, making it suitable for further development of an Mpox detection sensor.

6.5. Evaluation of Method Sensitivity



To enhance the sensitivity of the method, the research team evaluated the use of multiple sensors targeting different sites on the same DNA strand of Mpox. Three target sites in the approximately

190 kb DNA fragment of Mpox were selected. The increase in fluorescence signal generated by one sensor will be compared with that of three sensors. To determine whether these signals are linear and correlate with the concentration of the virus DNA, concentrations ranging from 0.5 to 250 nM were tested. At all concentrations, the group with 3 sensors showed fluorescence signals approximately three times higher than the group with only one sensor (Figure 10). With three sensors, the target DNA concentration as low as 0.5 nM can be reliably detected, while with just one sensor, the detection limit is closer to 2 nM. Therefore, low nanomolar DNA concentrations can be detected using only three sensors, and the sensitivity could be further increased by adding more sensors targeting other regions of the Mpox DNA.

7. Conclusion

This study successfully developed a fluorescence-based aptamer sensor method to detect the Mpox virus with high sensitivity and selectivity, addressing the urgent need for rapid and accurate diagnosis of infectious diseases. The SRhB aptamer was separated into two optimized oligonucleotide segments, which enhanced detection capability when encountering the target DNA. The optimization of buffer conditions, including pH, NaCl, and $MgCl_2$ concentrations, helped ensure an optimal environment for the reaction, improving fluorescence signal strength and sensor stability. The optimization of the incubation time between the aptamer segments and target DNA also minimized measurement errors and enhanced sensitivity, enabling detection of Mpox DNA at low concentrations.

During the selectivity testing, the fluorescence aptamer sensor demonstrated a strong ability to distinguish between DNA from the Mpox virus and similar DNA from other pathogens such as the chickenpox virus and bacteria causing skin infections. This capability is crucial to avoid misdiagnosis, ensuring effectiveness in contact tracing and treatment of Mpox.

Furthermore, the use of multiple sensors targeting different sites on the same DNA strand of the virus demonstrated a significant improvement in sensitivity. With a three-sensor structure, the method could detect target DNA concentrations as low as 0.5 nM, compared to a detection limit of 2 nM when using just one sensor. These results show the potential application of the fluorescence aptamer sensor method not only in detecting the Mpox virus but also in expanding to other viruses or pathogens, contributing to the development of rapid, accurate, and cost-effective diagnostic technologies.

This method has great potential for widespread use in public health diagnostics, especially in resource-limited areas where performing complex tests like PCR is challenging. The achieved results not only pave the way for developing aptamer-based biosensors but also highlight the promise of aptamers as tools in the

advancement of modern diagnostic technologies, meeting the growing demand for fast, efficient, and accessible healthcare solutions.

8. References

McCollum AM, Damon IK. Human monkeypox. Clinical infectious diseases. 2014;58(2):260-7.

2.

Gessain A, Nakoune E, Yazdanpanah Y. Monkeypox. New England Journal of Medicine. 2022;387(19):1783-93.

3.

Mitjà O, Ogoina D, Titanji BK, Galvan C, Muyembe J-J, Marks M, et al. Monkeypox. The Lancet. 2023;401(10370):60-74.

4.

Chowdhury PPD, Haque MA, Ahamed B, Tanbir M, Islam MR. A brief report on monkeypox outbreak 2022: historical perspective and disease pathogenesis. Clinical Pathology. 2022;15:2632010X221131660.

5.

Altindis M, Puca E, Shapo L. Diagnosis of monkeypox virus–An overview. Travel medicine and infectious disease. 2022;50:102459.

6.

McConnell EM, Nguyen J, Li Y. Aptamer-based biosensors for environmental monitoring. Frontiers in chemistry. 2020;8:434.

7.

Lu X, Kong KY, Unrau PJ. Harmonizing the growing fluorogenic RNA aptamer toolbox for RNA detection and imaging. Chemical Society Reviews. 2023;52(12):4071-98.

8.

VarnBuhler BS, Moon J, Dey SK, Wu J, Jaffrey SR. Detection of SARS-CoV-2 RNA using a DNA aptamer mimic of green fluorescent protein. ACS chemical biology. 2022;17(4):840-53.

9.

Gu L, Zheng J, Zhang Y, Wang D, Liu J. Capture‐SELEX of DNA Aptamers for Sulforhodamine B and Fluorescein. Chemistry–A European Journal. 2023;29(72):e202302616.