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Intermediate / Health

A Novel DNAzyme-based Colorimetric Assay for Early Disease Diagnostics under $10

In this project, a novel disease diagnostic assay was developed, applying DNAzyme technology, for the sensitive detection of pathogenic DNA in the blood. Current means of diagnosing diseases are extremely costly or unreliable. This simple assay allows for the highly sensitive, reliable yet also cost-effective detection of any pathogens for under $10, allowing for the widespread adoption of genetic-based diagnosis for all pathogenic diseases.

Project Forms

A Novel DNAzyme-based Colorimetric Assay for Early Disease Diagnostics under $10

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Introduction

Ever since the dawn of modern medicine, the early detection and effective treatment of both infectious and non-infectious diseases has been, and still is, one of humanity's greatest challenges.

In many nations, particularly developing, widespread diseases such as tuberculosis infect as much as one fourth of the global population [1], attributing to over 1.7 million deaths yearly. Even in developed nations, highly treatable diseases such as pneumonia cause the deaths of over 50,000 individuals annually [2].

Nevertheless, the critical problem of staggeringly high mortality rates lies not in the lack of effective treatment, yet in the extremely late diagnosis of countless diseases.

From cancer to HIV, malaria and syphilis, late diagnosis is the largest factor contributing to the loss of life around the world. In 2014, an extensive study done by the Centre of Public Health concluded that over a third of all HIV cases in Britain have been diagnosed too late, causing irreversible damage [3].

As a result, late diagnosis has continued to play an unimaginably large role in the high rates of mortality and proliferation of diseases globally, causing significant losses in global economy and life.

Project Objective & Abstract

Project Objective

The goal of reducing or even eradicating late diagnosis is only feasible through the development of an early disease diagnostic method that is cheap and simple, yet also accurate and accessible.

Unfortunately, existing methods for rapid early disease diagnosis are either exceedingly expensive or highly unreliable, and the development of a reliable, accurate yet cheap and widely available disease diagnostics method still represents a major challenge in modern medicine.

Thus, the end goal of this project was to fully address the current limitations in disease diagnosis technologies through the development of the ideal disease diagnostics assay. Such a diagnostics assay must be sensitive, reliable, yet also highly affordable and applicable for many diseases. This would be done through the integration of newly discovered novel molecular technologies.

Abstract:

This research project was concerned with the development of a method for the reliable, accurate and cheap early diagnosis of infectious diseases. The research project followed the scientific process closely, entailing three major sections: molecular computer simulations, lab experimentation and the construction of an innovative device.

Molecular Simulation: The goal of computer molecular simulations was to develop different approaches to use newly discovered DNAzymes (explained later on) for a simple yet powerful diagnostic assay. Four different assays were developed, simulated, and analyzed in order to find the one that best matches the required criteria.

Lab Experimentation: The best assay, identified through molecular simulations, was constructed and tested in the lab. The method consists of mixing a low amount of blood with an aqueous solution of gold nanoparticles and DNAzymes, and observing a distinct color change. Initially, due to the gold nanoparticles, the solution appears purple in color. Blood is then added in the solution, which turns distinctly red if pathogenic DNA is detected, allowing for the simple colorimetric detection of pathogenic DNA in the blood.

The goal of experimental testing was to determine the sensitivity and accuracy of the method, and confirm its applicability for the detection of low amounts of pathogens in the blood.

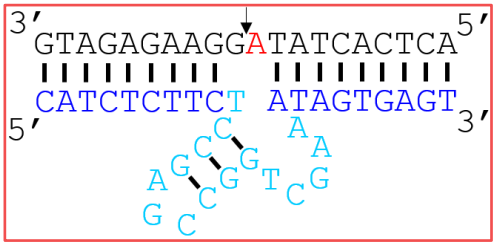
The results of the lab experimentation showed that the method's accuracy and sensitivity was unparalleled, while also allowing for the simple and rapid diagnosis of nearly all pathogenic diseases.

Device Development: As a final step, a portable, cheap and highly available device was developed to apply the method on a large scale. The device uses spectral analysis to determine the concentration of pathogenic DNA in the blood, and allows for the monitoring of the disease progression even before the appearance of symptoms.

Experimental Methods

Project Foundations

- The project chose to focus on the detection of pathogenic DNA in the blood as the method for diagnosing a wide spectrum of diseases. This method would allow any pathogenic disease to be detected.
- Next, DNAzymes were chosen as the main components for the detection of pathogenic DNA [4].



DNAzymes are DNA oligonucleotides that can be configured to bind to any genetic sequence, and when activated, they can cleave nucleic acid strands.

In the diagram, the blue/cyan section is the substrate arm, where target DNA binds (due to a complimentary genetic sequence) to the DNAzyme's binding mechanism.

The red section of the DNAzyme is the region of the nucleic acid that is cleaved.

Molecular Simulation

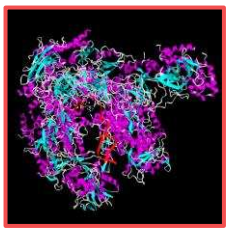
Numerous chemicals that showed promise for the development of a reliable diagnostics assay with a simple visual output were explored.

Four prominent chemical components – gold nanoparticles, sulphur molecules, lithium ions and protein macromolecules – were chosen. Each one was then incorporated to develop four disease diagnosis methods that were reliable and simple, with DNAzymes as the central component for detecting the DNA of pathogens.

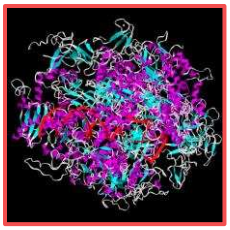
Using a molecular modelling and simulation software known as CHARMM [5] (Chemistry at Harvard Molecular Mechanics), each method was modelled and repeatedly simulated in order to provide a detailed and highly accurate comparison of all proposed diagnosis methods.

Method using gold nanoparticles as colorimetric agents to show catalyzation of DNAzymes.

Method using sulphur molecules to represent catalyzation of DNAzymes through viscosity change.



Method using lithium ions encased in a molecular shell for the heat-based diagnostic assay of pathogenic diseases.



Diagnostics assay using macromolecular proteins for the diagnosis of pathogenic diseases through opacity change.

For each simulation, CHARMM returned a vast array of data (i.e. running time for each simulation, detection time, rate of error, and accuracy). The best results for each method are highlighted in yellow:

	GNPs	Sulphur	Lithium Ions	RNA
Simulation running time	3h 42m	4h 21m	3h 58m	5h 06m
Analyte detection time	1h 22m	2h 05m	1h 49m	2h 50m
Rate of Error	0.015%	0.025%	0.018%	0.036%
Accuracy	98.9356%	96.928%	98.5739%	92.364%
Stability (nanometer deviations)	12nm	14nm	25nm	10nm

As the results clearly show, a GNP-based disease diagnosis method [6] is the optimal method for the rapid detection of pathogens. Thus, this was the method that was going to be developed in the lab.

Principles of the chosen method

The principles of the gold nanoparticles disease diagnosis method (that was chosen) are the following:

- Tiny amounts of blood are put into a test tube filled with an aqueous solution (initially purple in color)
- The initial aqueous solution contains GNPs that are binded in a vast network by nucleic acid strands. DNAzymes are also attached directly to the nucleic acids.
- When DNAzymes bind with the proper pathogenic DNA sequence, they catalyze and cut the nucleic acid strands that hold together the gold nanoparticles, separating the gold nanoparticles
- Due to their plasmonic properties, gold nanoparticles appear purple when held together but red when separated. As a result of their separation, the color of the aqueous solution changes from purple to red

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Lab Experimentation

In the lab experimentation, the chosen diagnostic method was built and tested in the lab. The objective of the testing was to determine the accuracy, reliability and sensitivity of the method.

Method Construction:

Preparation of aqueous solution with gold nanoparticles: 1 mL of 25 nM gold nanoparticles was added to 112 mL of nanopore water. Solution was boiled, making the solution colour turn red.

Attaching DNA linker strands onto GNPs: 75 µL of Agg thiol-functionalized nucleic acid strands were mixed with the aqueous solution containing the gold nanoparticles.

Solution was incubated for 20 minutes at 25°C, and then salt was added to allow loading of DNA on GNP surface. The binding of GNPs & DNA strands turned the solution from red to purple

Attachment of DNAzymes to current structure: 15 µL of DNAzyme solution was added to current solution along with MgCl2 (magnesium chloride) in a quantity of 4 µL, which helped bind DNAzymes with nucleic acid structure.

Testing for sensitivity

After the method's construction, random genetic sequences from different pathogens were chosen. Each genetic sequence was to be tested in the diagnostic assay at different quantities, from 0pM to 5nM. Through the insertion of these DNA analytes at the specified quantities (shown in the table below) with 2mL of blood, the sensitivity of the GNP-based disease diagnosis method was tested.

4. Target sequences	
AF-1[1]	CAG TGA CTT CAC ATG GGG CAA TGG CAC CAG CAC GGG CAG CAG CTG GC
Gon[2]	TGC TTA TTC TTC AGG TAC CGT CAT CGG CCG CCG ATA TTG GCA AC
Mal	AAA ATT AAG TGT TCA TAA CAG ACG GGT AGT CAT GAT TGA GTT CAT TGT GT
Syph	GGA GAC TCT GAT GGA TGC TGC AGT TGA CGT GTT TGC CGA TGG ACA G
HBV	GCC AAA ATT CGC AGT CCC CAA CCT CCA ATC ACT CAC CAA CCT CCT GTC CTC CAA

Using online tool GenBank, unique genetic sequences from several prominent diseases (Hepatitis B, Syphillis, Malaria, Gonorrhea, and Flu) were selected as target DNA sequences to be tested in the experimental disease diagnosis method.

This table shows the genetic sequences randomly chosen from different infectious pathogens, each of which was inserted in the assay at varying quantities.

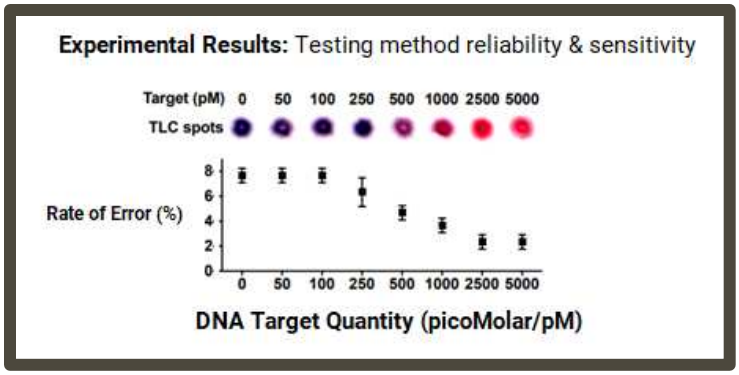
HBV	Syph	Mal	Gon	AF-1
5pM	5pM	5pM	5pM	5pM
10pM	10pM	10pM	10pM	10pM
50pM	50pM	50pM	50pM	50pM
100pM	100pM	100pM	100pM	100pM
500pM	500pM	500pM	500pM	500pM
1000pM	1000pM	1000pM	1000pM	1000pM
5000pM	5000pM	5000pM	5000pM	5000pM

Table showing the quantities of DNA analyte inserted for each genetic sequence

Results

The graph to the right displays the results of the experiment: the color change in the method as the amount of DNA target is inserted.

As the graph shows, the sensitivity is at around 300pM, which is when the color starts to change.



Many other leading diagnostic methods, such as PCR have a sensitivity of 200-500pM, meaning such a developed method can effectively rival the accuracy of well-establish assays, while also providing a method that is cheap and simple, widely available, and applicable to diagnosing nearly any pathogenic disease.

Conclusion

The goal of this project was to devise an innovative solution for one of the major challenges in modern medicine: the development of a method for early disease diagnosis that is sensitive, reliable, accessible and portable, simple to use and very cost effective. Through the combination of complex molecular simulations followed by in-depth lab experimentation, this newly developed assay proved to hold up as the ideal disease diagnosis method.

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- **Dr. Adam Shuhendler:** Professor at the University of Ottawa, at the Shuhendler Lab. Provided the permission for laboratory use and all necessary equipment for the lab experimentation stage of the project.

  Additionally, Dr. Shuhendler helped supervise the project experimentation as it was occurring in the lab. Nevertheless, everything that is described in the project report, from the molecular simulations to even all lab experiments were conducted and operated solely by the author, Albert Nitu.

  All results were also formulated solely by the author.

References

1. apps.who.int/iris/bitstream/handle/10665/259366/9789241565516-eng.pdf?sequence=1
2. www.cdc.gov/nchs/data/nvsr/nvsr66/nvsr66_06_tables.pdf
3. www.cph.org.uk/wp-content/uploads/2015/12/Late-HIV-diagnosis-rapid-evidence-review_final_covers.pdf
4. www.ncbi.nlm.nih.gov/pmc/articles/PMC5381262/
5. www.ncbi.nlm.nih.gov/pmc/articles/PMC2810661/
6. www.ncbi.nlm.nih.gov/pmc/articles/PMC4101904/