Team Results Document

[AUSense]

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Supervisor:
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[August 17, 2021]

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الجامعة الأمريكية بالقاهرة

SensUs

2021
Influenza

1. Abstract

AUSense has developed a conceptual biosensor with a great potential to detect and quantify hemagglutinin protein of the H1N1 influenza virus at low concentration ranges in human saliva; the amount of the measured hemagglutinin protein can then be correlated to the viral load present in the saliva sample. Driven by the extreme nature of COVID-19 pandemic, we focused on providing a robust and convenient testing device that serves as an essential and easy tool to control the spread of viral pandemics. The biosensor design is simple and doesn't require trained personnel. The proposed electrochemical biosensor detection is based on the use of antibodies and aptamers with high specificity to hemagglutinin protein, disposable screen-printed electrodes are used to test patients. The proposed biosensor concept is supported by molecular docking simulation to affirm the biosensor's ability to detect and quantify hemagglutinin protein within the desired concentration ranges. Moreover, we have introduced specific modifications in the biosensor which can adapt it to detect novel SARS-CoV-2 virus. We hope to further develop the biosensor prototype to provide a real-world contribution that makes our world more prepared for the next pandemic.

2. Biosensor system and assay:

The purpose of the developed electrochemical biosensor is to detect different concentrations of Hemagglutinin (HA) protein of the influenza H1N1 virus, which relies on the use of HA-specific molecules such as HA polyclonal antibodies and RNA aptamer for the detection. The biosensor consists of the screen-printed electrode and a potentiostat with impedance analyzer; the surface of the working electrode is functionalized with the RNA aptamer, which binds to the antibodies-functionalized magnetic beads, and the HA antigen. In the measurement protocol, immunoprecipitation is carried out in an Eppendorf by the addition of the antibody-functionalized beads and HA protein in phosphate-buffered saline (PBS, pH= 7.4), and l-cystine ethyl ester reagent is added and the tube is left to incubate for 1 minute [1]. The antibody-coated beads and antigen complex are magnetically separated in 10 seconds and the supernatant is removed; the precipitate is then resuspended in small buffer volume. Finally, the samples are pipetted onto the working electrode surface of the screen-printed electrode (SPE), which is connected to a potentiostat; the interaction of the aptamer and antibodies with HA protein induces a change in the measured current. The measurement protocol is outlined in Figure 1.

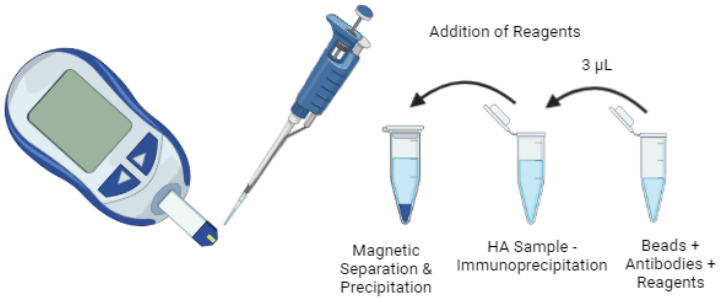


Figure 1. Biosensor prototype overview and measurement protocol

2.1 Molecular recognition and assay reagents

The surface of the working electrode is functionalized with RNA aptamer (Eurogentech, Belgium) that has a high affinity to HA [3]. The aptamer is thiolated at the 3' end using DDT reagent to allow for the chemosorption of the aptamer onto the gold surface of the working electrode through the formation of covalent bonds, resulting in a self-assembled monolayer (SAM) [5, 11, 12]. The immobilization process is carried out by the immersion of the electrode in reconstituted aptamer in PBS solution for 3 hours at room temperature, and then the electrode is treated with 6-mercapto-1-hexanol to block any remaining gold on the surface [2, 13].

The streptavidin-magnetic beads (Creative Diagnostics Inc., USA) are allowed to interact with biotinylated polyclonal HA antibodies (anti-bodies online, Germany) through streptavidin-biotin interaction forming antibodies-beads complex in an Eppendorf tube. A 3 µL of the antibody-beads solution is pipetted to the sample tube treated with 0.5 µL of l-cysteine ethyl ester reagent (concentration of 10mg/ ml), which will decrease the viscosity of saliva samples by disrupting the disulfide bonds between mucin found in saliva. The sample tube is then left to incubate for 1 minute at room temperature; the immunoprecipitation process can be expedited by the use of magnetic separators for 10 seconds to precipitate the formed complex out of solution [4]. The resulting precipitate can then be pipetted onto the surface of the SPE after calibration to determine the concentration of HA. The biosensor will be optimized to determine the LOQ, LOD and linearity range. The functionalized SPE surface is illustrated in Figure 2.

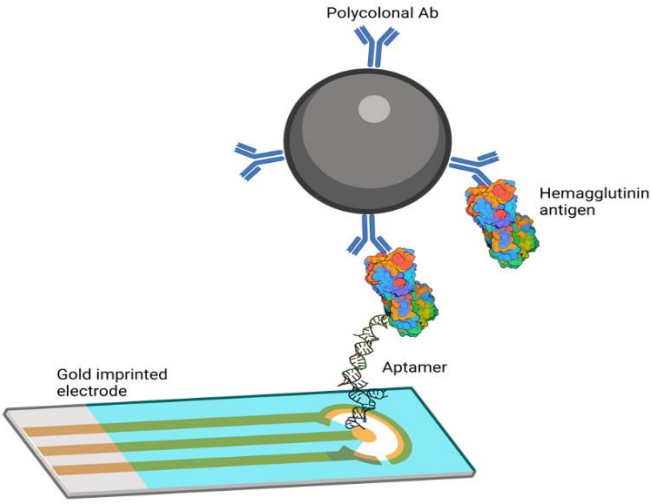


Figure 2. Functionalized Screen-Printed Electrode

2.2 Physical transduction

The electrochemical signal is transduced through the disposable screen-printed electrodes (SPE). The disposable SPE provided by PalmSens allows for both convenience and miniaturization of the sensors. The surface of the SPE is composed of gold, and Ag/AgCl for the reference and counter electrodes (WE: Au, RE: Ag/AgCl). When the aptamer binds to the antibody-bead complex and HA protein on the surface of the working electrode, the resulting electron transfer or change in current is detected. As the current passes between the working and counter electrodes, the reference electrode maintains a constant potential used as a reference for the working electrode. The SPE is connected to PalmSens4 potentiostat (provided by PalmSens, The Netherlands) which supports many types of voltammetry techniques as well as electrochemical impedance spectroscopy, in addition to the low current range the 100 pA to 10 mA, which all allows the biosensor to have a low limit of detection for HA protein.

2.2 Cartridge technology

The biosensor mainly consists of the disposable SPE coated with gold (PalmSens, The Netherlands) connected to a PalmSens4 potentiostat (PalmSens, The Netherlands). The main procedures involving fluid handling are the immunoprecipitation step, and the pre-treatment of samples with l-cystine (mucoactive agent), all of which are carried out in Eppendorf tubes. During immunoprecipitation, the formed complex is separated using magnets to allow for faster binding and separation. PBS solution is also used to calibrate the biosensor. The buffer and treated samples are to be pipetted onto the working electrode surface of the SPE.

2.4 Reader instrument and user interaction

The size of the biosensor hardware is approximately 15.7 x 9.7 x 3.5 cm^3 which is suitable for the use in point-of-care health monitoring. The patient will be able to handle the fluids according to the protocol, by mixing pre-determined volumes together for the sample pre-treatment and immunoprecipitation processes; the fluid is then added on the SPE surface for measurement. The user interface includes a PSTouch mobile application that can wirelessly communicate with the potentiostat. The mobile application interface will allow for the measurements of HA concentration using the calibration curve, in addition to serving as a platform for storing the patients' measurement history that can be shared over a cloud with their primary care physicians.

3. Technological feasibility:

3.1 Computational simulation

We used polyclonal antibodies that binds to amino acids between 1 and 343. We performed modelling for an aptamer structure from literature after contacting authors. We made a 2D model for the aptamer using Vienna tools (version 2.0) [9]. We built the 3D model by RNAComposer (version 1.0) [6]. Finally, we performed docking with the antigen using Hex (version 8.0.0) [10], software as in Figure 1., Hemagglutinin PDB ID is 3HTO [8].

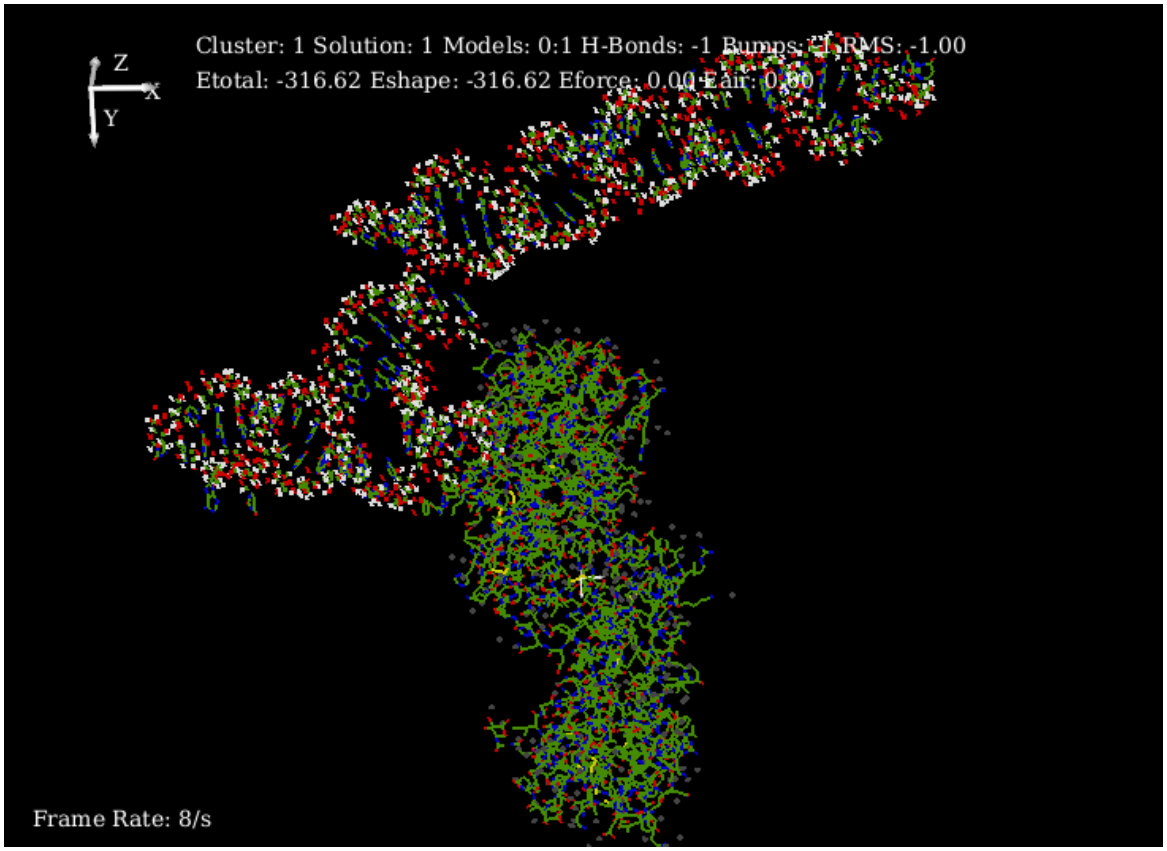


Figure 1, The upper structure is the aptamer represented in stick and ball form while hemagglutinin is represented as wire form. E. total is -316.62 which represents successful docking

3.2 Concentration calculation strategy

Different concentrations of the antigen will be plotted vs recorded electrical signal. An unknown concentration can be measured using the equation of the plotted line or curve.

3.3 Biosensor Limitations and proposed optimization

The main drawback of our biosensor is the fluid handling due to the use of two biological molecules in enrichment and detection. Thus, the main drawbacks are the use of multiple reagents and an antigen extraction kit (antibody functionalized magnetic beads) which is required for antigen enrichment.

Our biosensor can be optimized by adding a microfluidic cartridge to automate the process and decrease the human interference with testing. An alternative for the he multiple reagent problem is using a pre-filled syringe with the enrichment kit that is allowed to mix a fixed amount of the sample and apply it directly on the imprinted electrode.

For signal enhancement, we can redesign our recognition molecule to be an aptamer attached to an amplifier molecule (branched DNA) or a tag that can perform stronger and faster redox reaction [7].

For optimizing the measurement instrument, we can use a smaller size device like EmStat Pico Development Kit (PalmSens), which will have an advantage of directly connecting to a smartphone device. However, this will affect the sensitivity of the biosensor unless we amplify the electric signal by modifying the aptamer.

3.4 Intact virus proposal

We suggest that our biosensor will work better for intact virus if the concentration of the aptamer decreased for hindrance on the imprinted electrode. Therefore, our biosensor won't need major modifications for intact virus.

4. Originality

4.1 Team:

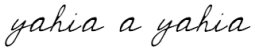
There are many types of developed biosensors that utilizes antibodies and aptamers for the detection of viruses and bacteria. Our team's objective was to develop a prototype, not only for the detection, but for the quantification of hemagglutinin protein at very low concentration ranges, thereby providing an early detection of viral infections. We have conducted an extensive literature review to come up with the best approach and strategy for the quantification of Hemagglutinin. We thought about having an approach that combines several molecules with high affinity to hemagglutinin protein, in addition to strategies that can enrich the antigen concentration to allow for low limits of detection and quantification.

We also reviewed many published protocols for the thiolation of aptamers, biotinlyation of antibodies as well as the preparation of magnetic beads linked to streptavidin. The aptamer sequences used are based on literature review; we contacted the first authors to confirm the validity of the aptamer sequences and its high affinity reported in their research papers. Furthermore, we investigated many techniques for enrichment of the hemagglutinin antigen and finally came up with the use of magnetic beads which is more time-saving compared to the use of ultracentrifugation. Since the needed components we ordered could not arrive on time, we needed to provide models that can support our biosensor concept.

4.2 Supervisor:

The team have rigorously reviewed the literature and investigated many strategies for the biosensor, including the use of lateral flow assays until they came up with the way to combine the use of antibodies and aptamers together, in addition to a novel method for enriching hemagglutinin that significantly reduced the analysis time. Despite the great challenges they faced due to the imposed COVID-19 pandemic, as well as the challenges in importing the needed reagents and time constraints, the team have managed to develop a conceptual biosensor and independently created molecular models to sustain their biosensor concept. In addition, they also managed to consult many experts from PalmSens and Medtronic for the feasibility and development of the biosensor.

To the best of our knowledge, the team developed an innovative biosensor concept with a novel approach to combine the use of aptamers and antigens and to address antigen enrichment for low limit of quantification while providing accurate results in a short period of time.

Supervisor:	Team Captain:	Team Captain
Dr. Hassan Azzazy	Bishoy Abib	Yahia Ahmad
		

5. Translation potential

5.1 Business model canvas:

<table>
<tr>
<td rowspan="2">Partners
1- Suppliers:
• PalmSens
• Qiagen
• Merck
• Sino Biological
• Eurogentech
• AgiTech Egypt on behalf of Sigma-Aldrich

2- Sponsors: AUC Research Funding.

3- Hospitals and medical centers.

4- Government organizations (Ministry of Health, Ministry of Tourism).</td>
<td>Key Activities

• Development and Production of the biosensor.
• Advertising for the Biosensor
• Finding Physicians, pharmaceutical companies, etc. through social media and networking using Facebook and LinkedIn
• Planning for expansions via market studies.
• Obtaining IP licensing and maintaining it.</td>
<td rowspan="2">Value Proposition

• Innovative concept and chemistry.
• Low cost compared to PCR tests.
• Higher sensitivity and fast testing.
• Portable device with point-of-care and IoT-based technology.
• Minimum sample handling.
• Time-gain for doctors due to the easiness and rapidity of the diagnosis, leaving more time for treatment.</td>
<td>Customer Relationships

• Sales touchpoints.
• Through physicians and diagnostic laboratories to identify patients.
• Through government for infection control in airports booths.</td>
<td rowspan="2">Customer Segment

• Healthcare Facilities.
• Hospitals.
• Airports.</td>
</tr>
<tr>
<td>Key Resources

• Human resources: R&D, labour and IT
• Intellectual resources: Patents via IP license and copyrights.
• Physical resources: raw materials and laboratories</td>
<td>Channels
• Commercial labs
• Government organizations
• Hospitals.
• Medical Sales Representatives.

• Online ads on websites and social media.
• Doctors’ offices and clinics.</td>
</tr>
<tr>
<td colspan="3">Cost Structure

• Cost of device materials
• Market studies
• R&D
• Customer service
• Lab Facilities
• Software Purchase/subscription
• IP and rights costs.</td>
<td colspan="2">Revenue Streams

• Initial funding from AUC
• Product direct sales (test strips)
• Licensing fees</td>
</tr>
</table>

5.2 Market description:

Egyptian market is a large market in a country of more than 100 million people. Egypt was visited annually by over 12 million tourists with an average of 1 million tourists per month. The number decreased by the pandemic and after decreasing restrictions it reached an average of 580 thousand tourists per month between January and June 2021. Therefore, controlling infectious disease is an essential for the benefit of tourism and decreasing the burden on the healthcare system. Our targeted customer will be airports and hospitals.

5.3 Stakeholder desirability:

We will target two main segments of customers in Egypt: Hospitals and airports. For hospitals, the burden left behind spreading an infection in a hospital is the main need for developing a fast point-of-care system for screening for influenza especially in Winter before the patient is admitted. In 2021, Egypt has about 1782 hospitals and over 1500 ambulance centers. A study about the infection of influenza after admission showed that 9.2% of influenza patients acquired it from the hospital with mortality rate of about 39% [14]. For airports, our target would be the Ministry of Tourism. Their need will be based on the fact that the WHO recommended screening tourists more efficiently since Egypt receives over 12 million tourists annually from all continents [15]. A spread of infection from Egypt will be a crisis on tourism while a tourist can feel safer when there is a testing confirming his healthcare when coming to Egypt.

In terms of value proposition, the alternative for our biosensor is serological testing and PCR. This needs an equipped lab with expert personnel. The cost that we are competing with is the wages of professional workers plus PCR kits required for the test. The initial cost of a thermocycler is over $4900 [16]. PCR tests can cost an average of USD 75 in COVID-19 for instance [17]. However, our test can cost under $35 and have the advantage of being point-of -care to be more accessible in different occasions and in ambulance for infection control. In addition, it is easier to use and does not require technicians over PCR which needs a technician whose wage can reach USD 350 per month.

5.4 Business feasibility:

The main activities of our company will be selling the device and its electrodes plus training for good practice and support. Our research and development team will work on developing the bioassays for improving the device's precision and accuracy as well as reducing the reagent's costs. The team will also collaborate with software engineers and programmers to provide the user interface and the applications necessary to integrate the biosensor into the IoT. The potential partner is PalmSens where they are going to provide the potentiostat devices and the screen-printed electrodes. The AUC will provide technical support and consultations in the initial years for the development of the biosensor components, thereby significantly reducing the manufacturing costs, and paving the way for commercialization. As for the marketing of the biosensor, we will start by awareness campaign in airports as brochures and online advertisements. We will target Cairo and Giza only at the first year. We will supply a device for two academic hospitals for the largest medical universities in Egypt (Al-Kasr Al-Einy hospital and Al-Demerdash), and for airports as well.

5.5 Financial viability:

The cost of the assay using the biosensor is approximately $10.2. We plan to decrease the initial assay cost through our research and development team and technical advisory boards, also by developing our biosensor using the EmStat Pico Development Kit. We are targeting to sell 1 strip

per bed and provide 30 potentiostat devices for free in the first year to cover the 30 public hospitals and clinics in Cairo and Giza. The 30 hospitals contain about 1119 beds. We will target 3 strips per bed per month of total 3356 strips. The strip will be sold at $16. We are going to expand our production capacity to cover over 26380 beds in 246 public hospitals and units and will target more airports after three years and scaling up. By providing the potentiostat for free, we aim to gain our profit margin through the sales of the test strips. N.B. All statistics are according to Egyptian Central Agency for Public Mobilization and Statistics (CAPMAS) issued article "Annual Bulletin of Health Services Statistics" in February 2021.

Our net profit margin will reach up to 24.9% of the total cost per year (total cost per year $432750, and the net profit will be $106050). Accordingly, our Profit statement for first year will be as follows:

AUSense

Profit and Loss (P&L) Statement

[USD]

	2018												
	JAN	**FEB**	**MAR**	**APR**	**MAY**	**JUN**	**JUL**	**AUG**	**SEP**	**OCT**	**NOV**	**DEC**	**Full Year**
Devices (550 per device)	-	-	16,500.0	-	-	-	-	-	-	-	-	-	16,500.0
Strips (12 per strip)	600.0	-	40,320.0	40,320.0	40,320.0	40,320.0	40,320.0	40,320.0	40,320.0	40,320.0	40,320.0	40,320.0	403,800.0
Returns, Refunds, Discounts	600.0	-	-	-	-	-	-	-	-	-	-	-	600.0
Total Net Revenue	**1,200.0**	**-**	**56,820.0**	**40,320.0**	**40,320.0**	**40,320.0**	**40,320.0**	**40,320.0**	**40,320.0**	**40,320.0**	**40,320.0**	**40,320.0**	**420,900.0**
Cost of Goods Sold	-	-	-	-	-	-	-	-	-	-	-	-	-
Gross Profit	**1,200.0**	**-**	**56,820.0**	**40,320.0**	**40,320.0**	**40,320.0**	**40,320.0**	**40,320.0**	**40,320.0**	**40,320.0**	**40,320.0**	**40,320.0**	**420,900.0**
Expenses													
Advertising & Promotion	550.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0	2,200.0
Devices (500 per device)	1,500.0	15,000.0	-	-	-	-	-	-	-	-	-	-	16,500.0
Strips (10 per strip)	500.0	33,600.0	33,600.0	33,600.0	33,600.0	33,600.0	33,600.0	33,600.0	33,600.0	33,600.0	33,600.0	33,600.0	370,100.0
Office Supplies	-	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	550.0
Rent	-	-	-	-	-	-	-	-	-	-	-	-	-
Technicians	1,200.0	1,200.0	1,200.0	1,200.0	1,200.0	1,200.0	1,200.0	1,200.0	1,200.0	1,200.0	1,200.0	1,200.0	14,400.0
Medical Representatives	1,200.0	1,200.0	1,200.0	1,200.0	1,200.0	1,200.0	1,200.0	1,200.0	1,200.0	1,200.0	1,200.0	1,200.0	14,400.0
Initial R & D cost	9,100.0	-	-	-	-	-	-	-	-	-	-	-	9,100.0
Lab supplies		500.0	500.0	500.0	500.0	500.0	500.0	500.0	500.0	500.0	500.0	500.0	5,500.0
Total Expenses	14,050.0	51,700.0	36,700.0	36,700.0	36,700.0	36,700.0	36,700.0	36,700.0	36,700.0	36,700.0	36,700.0	36,700.0	432,750.0
Earnings Before Interest & Tax	**(12,850.0)**	**(51,700.0)**	**20,120.0**	**3,620.0**	**3,620.0**	**3,620.0**	**3,620.0**	**3,620.0**	**3,620.0**	**3,620.0**	**3,620.0**	**3,620.0**	**(11,850.0)**

6. Team and support

6.1 Contribution of team members:

Our multidisciplinary team consists of three graduate and three undergraduate students

Yahia Ahmed (Biotechnology), Salma Aboul-Hassan (Biology), Bishoy Abib (Chemistry), Iman Mostafa (Biotechnology): Conducted the Literature review, in addition to working on finding aptamer sequences, molecular docking models, thiolation and immobilization of aptamers, conjugation chemistries of antibodies and immunoprecipitation, ordering the needed components and reagents, in addition to conducting surveys with medical professionals.

Salma Soliman (Electronics Engineering), Andrew Hany (Computer Science): Mainly worked on the physical transduction part, selection of suitable potentiostat, reader instruments and electrodes, designing the user-interface and experience, in addition to working on the translation potential and applying for undergraduate research grants from AUC.

6.2 People who have given support:

Professor Hassan Azzazy, Distinguished Professor of Chemistry and Chairman, AUC

Dr. Hassan was the main supervisor, who was responsible for ensuring the progress of the team as well as providing technical and financial support through regular meetings and securing grants for the biosensor project. In addition, he was providing support to facilitate our reagents' orders and import procedures.

7. Final Remarks

This was a remarkable experience. We are grateful to the organizers for their efforts and cooperation throughout the year of the competition. We have learned tremendously and made lots of new friends.

8. References:

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