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Exploring the route choice of slime mold in a ballistic logic gate maze in different conditions

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Abstract:
This study investigates the factors affecting the smart behavior of slime mold, a single-celled simple organism, in a ballistic logic gate maze. The slime mold can choose between the two output sites marked by food and act as a decision-making logic gate that can be applied in biological computing and other logic-requiring operations. The molds were hypothesized to have direction-determining abilities, which allowed them to choose a straight path starting from a dead-end and grow without turning until another dead-end was reached. Experiments as primary research are conducted for six groups of slime mold, varying in food type on the output site, and the stress level of the mold is determined by the fed/unfed status. Incorporating evidence from time-stamped photographs recorded over a 20-hour period, our analysis shows a strong correlation between the speeds of growing/food covering and the slime mold's stress level. We concluded that the unfed slime mold grows slower on agar gel but covers the food faster and thus has a greater overall speed in completing the logic gate. The speed of covering the food is the fastest on oats, followed by yogurt, and is the slowest on apple slices. The results of the success rate regarding the straight-through growing behaviour of the slime mold are lower than expected. While accessing limited sample size during the experiment, this study questions the reliability of the slime mold direction pattern and recommends further research and confirmations on the issue.

Keywords: *Physarum polycephalum*, ballistic, logic gate, unconventional computer

1. Introduction

The plasmodial slime mold *Physarum polycephalum* is an amoeboid organism that mainly consists of net-like yellow structures and slime-texture routes (Nakagaki and Yamada, 2000). Its plasmodium containing

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thousands of nuclei is not divided by cell membranes and exists as one single cell. The routes expressed by its cells are in the form of a specific and sufficient network. During its growth, regular fluctuations and optimizations of different routes have been observed (Nakagaki and Yamada, 2000). This unique phenomenon consisting of intelligence without any complex neuron cells or central control system has been adapted to route choosing, railway map designing, and even computer chip making.

Slime molds can be found in wet areas, common in damp forest soils, tree bark, and dead or dying wood (Ing, 1999). Having multiple nuclei in a single membrane, the slime mold exchanges its exploration signals through the plasma cell in the form of fast-moving calcium ions. Once a route is explored and does not reach any food source, the route is then disposed of and leaves a slimy path to record the result of failed exploration. The future explorations would then recognize this slimy signal and move to other directions instead of demonstrating repeated paths.

While capable of completing complicated tasks, the growing mechanism of slime mold is simple and low energy-demanding, compared with contemporary computer programs and artificial intelligence (Douglas, 2024). It can output patterns with the most sufficient route connecting its food, based on the input of slime mold locations and the maze setting. Though it might be time-consuming, it often requires 24 to 48 hours of growing across a medium (Halvorsrud and Wagner, 1998), the idea of having intelligence without a brain is revolutionizing the current impression on the formation of thoughts and consciousness.

The focus of this study is on the combination of slime mold and the logical expressions made by computer programs. During its growth, the slime mold can demonstrate a logic gate in mainly two forms. The first is the logical choice of whether to grow in a certain direction or not when exploring and optimizing its path, often expressed by solidified and dim slimy routes that are identified under a light microscope. The second is the overall path expressed by the slime mold after optimizing and performing a route visible to the naked eye (Adamatzky, 2015). In the second type of logic gate, the slime mold performs the characteristic of traveling in its determined direction until a food source is found or a dead-end is reached. If the slime mold starts growing in a one-end path, after exploring all the directions, it will grow in the only direction without blocks, ignoring further existing turning points available on its way, until this single direction has been completely explored (Adamatzky, 2010). This characteristic can be implied in many designs including functional mazes like the logic gate.

This study aims to set up a manageable slime-mold-functioned ballistic logic gate by demonstrating the smart behaviour of slime mold, and by studying the principles of a computing ballistic gate. Concluding from previous research on the slime mold, this amoeboid organism is grown under successful conditions and can perform a logical choice based on its route-optimizing actions in the setting of occasions represented by the presence of slime mold and oat pieces. The resulting ballistic gate can act as a model for more complicated logic program demonstration by expanding the number of input and output sites. During the experiments, the mechanism of biological intelligence of the slime mold will also be investigated.

2. Methodology

2.1 Data Collection

The research conducted to support this dissertation includes primary and secondary research. Secondary research is mainly carried out on Google Scholar and some official websites of universities and organizations. The research covers the topics of slime mold growing, past research on the smart behaviour of the slime mold, and logic gate programming. The information and data are collected on a reading log and the reliability of the contents is analyzed by CRAAP (currency, relevance, authority, accuracy, purpose) test (Blakeslee, 2004).

Primary research is the experiments this study included regarding the previous experiments on this subject. Firstly, the pre-experiment was designed to adapt to the slime mold's growing cycle and characteristics, such as its optimal temperature and the amount of food required to grow across the whole medium over a certain period. The slime mold was grown on agar gel (4.8 g/dm^3, strength 1700 g/cm^2). A few experiments are based on the phenomena studied by past research, consisting of growing process recording and simple maze-solving. Fresh oat pieces (bought from Taobao) were given once a day, in the amount of five pieces at a time. The final determined growing conditions for the slime mold are 25 ℃, oat-fed, and treated with dim light that allows photographing without significant impact on slime mold growth. Then, molds are grown under these conditions as the "fed" group for the formal experiment.

Secondly, this study conducted a series of experiments to set up a slime mold ballistic logic gate with a high correct result rate, utilizing this organism's optimization skills. The ballistic gate consists of two input sites and two output sites, with four different scenarios of input and output respectively (Adamatzky, 2010). Upon placing the slime mold in an input site, it starts to grow and explore the surrounding routes, finding all but one being dead ends, and grows in that corresponding direction. The pathway formed by slime mold exists as a recognizable single pathway that can be identified easily by observation. The presence of slime mold at a particular site which is either input or output represents “1”, and the absence of slime mold represents “0”. The two connected pathways that the slime mold can choose are set so that the mold can grow in certain directions according to its direction-determining character. The maze designed for this experiment is modeled on a computer and printed by a 3D printer to ensure that the mazes of all different experiments are the same (Fig. 1.1). The path of the maze is made by agar gel exported from the 3D model, to minimize the effect of the 3D printing material on the growth of the slime mold, with average diameter of 0.9 cm on the outside (include walls) and 0.65 cm on the inside (without walls).

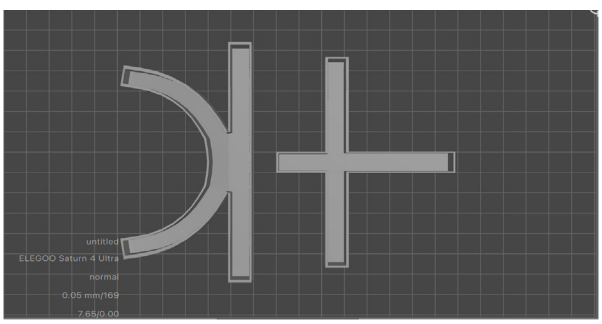

Figure 1 3D diagram of the logic gate maze, showing “k” shape (left) and “t” shape (right)

There are six groups in total in the formal experiment, with eight trials in each. Out of the eight trials in one group, four trials are taken on the “k” shaped maze, and the other four trials are taken on the “t” shaped maze. Different conditions including the stress level determined by whether the food fed to the slime mold before it is placed in the input site (fed by oats for 24 hours before the experiment), and the type of food (oat, yogurt, or apple) present on the output site of the logic gate, are applied to molds of different groups that have the same maze setting and the same input. Each output site is applied with a single piece of oat, and the yogurt (for every 100 g, this yogurt contains 266 kJ energy, 2.6 g proteins, 1.4 g lipids, 10.0 g hydrocarbons, 60 mg sodium, and 80 mg calcium) and apple are applied in the area of the output site is similar to oat. The “fed” groups of slime mold are transported from the previously grown mediums, and the agar gel with mold close to oat pieces is cut as whole and placed on the input site of the logic gate. All groups of slime molds are placed initially in site “input 2”. To determine a “success”, the two output sites are labeled as “p” site and “q” site, and for both maze shapes, the “success” site is the “p” site, while all other resulting routes (reached the other site, reached both sites, or reached neither) are counted as a “fail” (Fig. 1.2 and Fig. 1.3). The amount of time which slime mold takes to reach any output site is recorded by a time-lapse camera at a five-minute interval to investigate the efficiency of slime mold logic gates. The total observation time for each group ranged from 20 hours to 24 hours, and the final analysis of the food coverage was based on the last photograph taken at the 20 hours stamp.

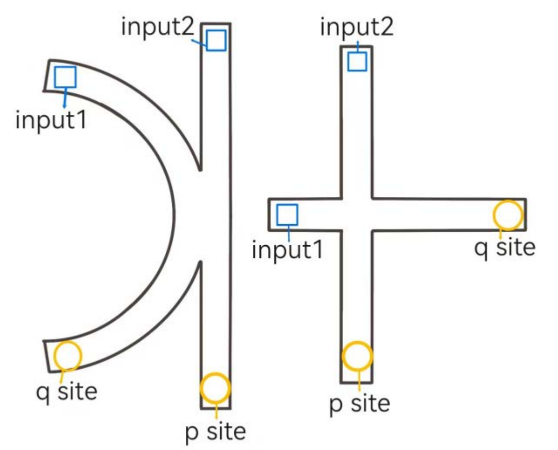

Figure 2 The input and output sites of the ballistic logic gate

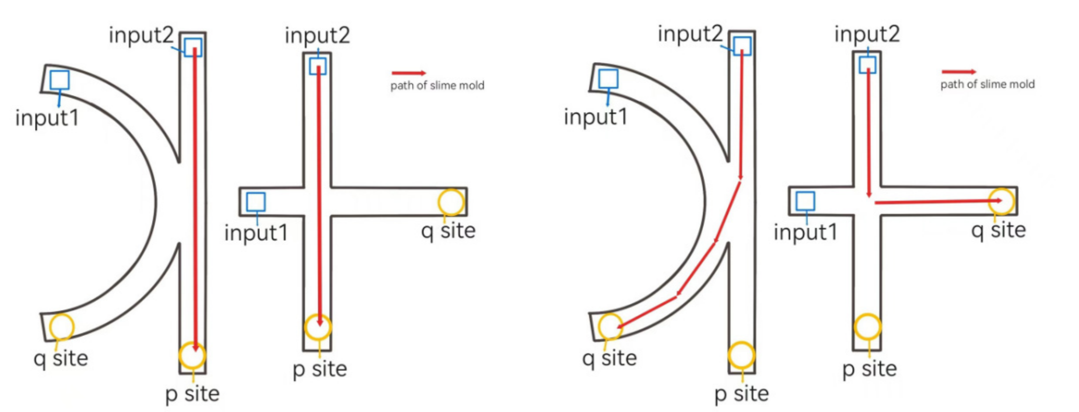

Figure 3 The scenarios of the ballistic logic gate, with “success” (left) and “fail” (right)

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2.2 Data Analysis

The success rate is calculated by: (number of success scenarios) / (total number of mold gates being operated). And according to the recordings made by time-lapse filming in a setting of one shot every five minutes, the time taken for the slime mold to reach the food site can be estimated. Taking an average of the recorded time of the groups, the photograph at 20 hours is used to determine the food coverage of the output site, marked on the coverage index from 0 to 4, in which 0 shows no coverage on food, 2 shows half coverage, and 4 shows full coverage (Fig. 1.4). The reaching time and the food coverage both exhibit the growing speed of the slime mold, on agar gel and on food sites, respectively.

0 1 2 3 4

Figure 4 Food coverage scale from 0 to 4

The collected data will be analyzed in combination to determine the conditions that yield the greatest success rate and optimal growing time. These data can together show the feasibility and applicability of the slime mold ballistic logic gate under certain stress levels and food sources.

3. Literature Review

3.1 Introduction - Slime mold discovery and its features

In the 20th century, the *Physarum polycephalum* branch of slime mold was first identified and studied. It is observed that approximately fifteen to seventy-two hours after the application of water to the slime mold spores, the life cycle of the non-toxic mold began with the cell walls splitting and the nucleus dividing mitotically (Howard, 1931). In laboratory conditions, the observations usually start with sclerotia of the slime mold which were originally kept in dry and cool conditions. This can reduce the activation time of this mold to a time close to its regular fluctuation frequency, for a sclerotium only needs to be waken compared to a full life cycle starting from a spore. The discovery of slime mold contributes to later studies on choice-making, intelligence generation, and cellular computation, which are critically significant for today's artificial intelligence era.

The growing pattern of slime mold is recorded to be around 1 cm/h with its locomotion through fluctuations on the agar gel, resulting in a giant, unicellular structure that includes multiple nuclei and can be seen by the naked eye as a yellow organism consisting tubules of differing thickness (Tero *et al*, 2010). The thickness of the tubes indicates the importance of a certain route, showing whether the route has been disposed of, determined, or is still in progress. Inside the cell, the organs are densely connected and arranged by components identical to microtubules (Nakagaki and Yamada, 2000). The unicellular combination makes it possible and convenient to conduct intracellular mineral transplantation through its tubule structures.

3.2 Smart behaviour of slime mold

By the year 2000, the Japanese researchers Nakagaki and Yamada found that this amoeboid organism had a special way of growing which can make choices of its path, resulting in behaviours involving intelligence. Their study further discovered that the slime mold could optimize routes connecting the food sites with which it was provided. This phenomenon was used to re-design a railway map of Tokyo city, using oat flakes of different sizes as major and minor stations, and light regions applied as mountains, rivers, and other constructive obstacles. The slime mold is left to grow freely over a 26-hour period, and after comparing it with the real image of the Tokyo railway map, the experiment resulted in a yellow route highly identical to the one designed and constructed by human engineers, with even some improvements (Tero *et al*, 2010). The discovery opened up a new approach to intelligence and showed an alternative way to optimize routes and make choices.

3.3 Mechanism

During its growth, the slime mold undergoes a series of mechanisms in response to environmental interactions. This mainly involves oscillations produced by the transport of calcium ions and some other cellular minerals. These oscillations are chemical communication between different sections of the slime mold, resulting in a physical expression (Boussard, 2020). The frequency of the oscillations and the feedback it receives from the environment including food, predators, and nothing within a certain region away from the main exploring point, determines whether to continue growing in one direction or turn to another (Boussard, 2020). With the fluctuations in its structure, the slime mold can explore its surroundings and choose its final routes. After exploring a region that has no connecting sites, such as without food sources, the slime mold will retreat from that certain direction and leave a slimy path as a sign of already-explored, after which further explorations will not reach that way (Nakagaki and Yamada, 2000). The transport of signals and the marking of slimy paths can both serve as the mechanism when the slime mold finishes its exploration and starts to optimize its route. If various food sites are given, the slime mold will gradually connect the sites with a most sufficient path, corresponding to the "traveling salesman problem" solved with comparatively complicated calculations on a computer.

The route-choosing and memorizing abilities of the slime mold are influenced by various factors, most of which are related to the slime mold's growth capabilities. Temperature, humidity, the level of calcium ions and ATP (Yoshimoto and Kamiya, 1984), and the different food sources are some of the factors involved. 25°C and relatively damp environments are the most suitable for slime molds, and barley-fed slime mold is observed to grow the fastest out of barley, oats, and quinoa (Rawshan and Davidoff, 2023).

3.4 Slime mold and the logic gate

With its special smart behaviour that requires only simple mechanisms, the slime mold has been applied to some other smart aspects including computing. To design a smart computing system using slime molds, the logic gate is a fundamental and essential component. It makes output choices based on different inputs, such as "yes" or "no", "1" or "0", to determine whether an event occurs (Jaeger, 1997). The combination of a logic gate series can run complex programs, which is the building block of a computing system.

The studies on slime mold and logic gates have split into two main directions: the slime mold's logic in choosing routes during exploration, and the overall expression of its route once exploration is complete. Both use the optimizing feature of slime mold, defining different input and output settings. Inside a slime mold, the tubes reach out to find food sources and can perform output by determining whether a food site is reached or not. If a food site is reached, the tube connecting that particular site will continue to grow, outputting a "yes". If not, the tube will return and can be considered as an output of "no". For inside the slime mold, the returned tube appears as a faded trace of slime, while the determined tube will continue to appear yellow and conduct fluctuations during cellular transport. For the overall expression, the slime mold will determine a successfully discovered route that is yellow and relatively thick, for some time before it continues to explore other parts.

3.5 A ballistic approach

For the second direction of slime mold logic choosing, though some forms of the logic gate are difficult to be carried out by slime molds, the ballistic approach to the logic gate is based mainly on positive choice making, with two inputs and two outputs. Studies concluded that the slime mold can demonstrate ballistic behaviour when placed in a crossing maze, moving in one direction it determined earlier and briefly ignoring other turnings even when accessible (Adamatzky, 2010). With this feature, a logic gate composed of agar gel can be designed for slime mold to grow. In this study, the only food source used on the output sites is oats.

3.6 Limitations and possible improvements

According to the latest research on ballistic gates run by slime molds, the reliability associated with the success rate of the gates is relatively low (Adamatzky, 2010). Regarding the various environmental factors affecting slime mold growth and memory, this study focuses on two of the factors with a high likelihood of affecting the overall performance of the slime mold ballistic gates, the stress level of slime mold and the type of food provided on output sites. Furthermore, this study includes another dependent variable to be observed, the time taken for the slime mold to reach an output region, and the growing speed on the output site, further stating the efficiency of the logic gate. These improvements may elaborate on previous studies, suggesting a more predictable slime mold logic gate under certain conditions.

4. Results

Over an observation time over 20 hours, the site reached and the area covering the food are recorded as data below. Taking on average, the already-fed slime molds were faster to start growing and to reach the ending site, while the new slime molds grown from sclerotia needed time to wake and took longer to finish the maze (Fig. 2.1).

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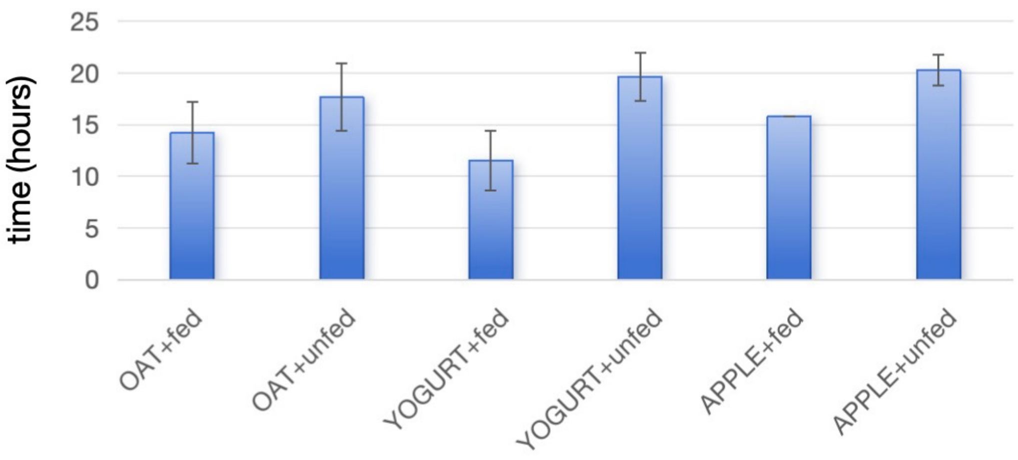


Figure 5 The average time taken by Physarum polycephalum to reach a food site

The average coverage of food was estimated using photographs taken at the end of each experiment, and it showed a different pattern. Although they took longer to reach the food, the already-fed slime molds resulted in less coverage compared to the slime molds woken from sclerotia. For the food provided, upon reaching the food site, oats are the fastest to be covered, followed by yogurt, and the least covered food is sliced apple (Fig 2.2).

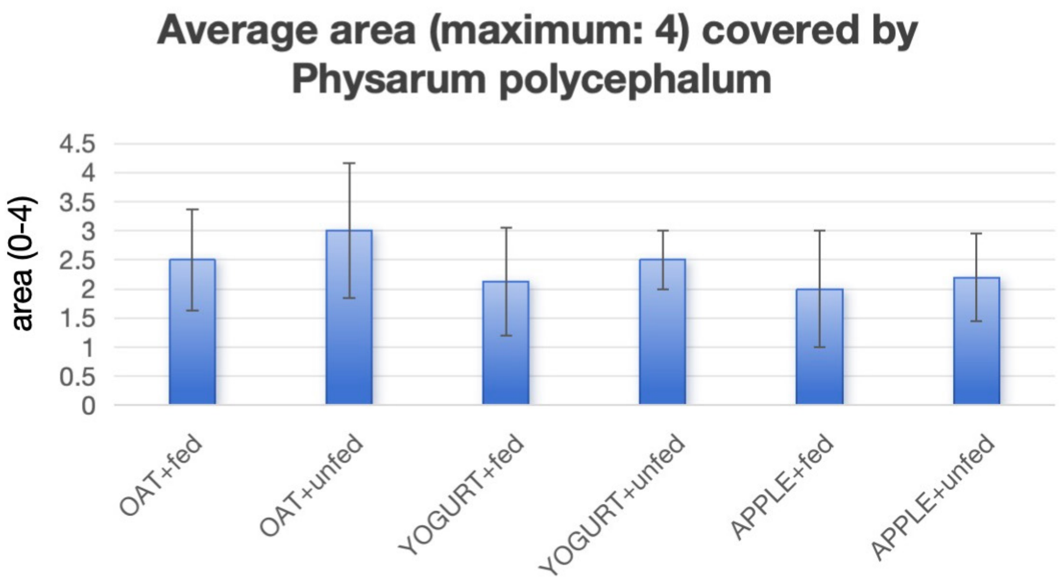


Figure 6 The average area (maximum: 4) covered by Physarum polycephalum at the end of experiment (20 h)

The site considered to be "success" for both maze settings of different shapes was the "p" site, and the calculated success rate is relatively low for all groups in the experiment (Fig. 2.3a). A proportion of slime molds split into two directions and reached the "p" site and the "q" site at the same time, and this particular scenario was counted as "fail" as well. The average of the overall success rate for the "k" shaped maze was 37.5% (±0.10), slightly higher than the "t" shaped maze, which was 29.2% (±0.16) (Fig. 2.3b).

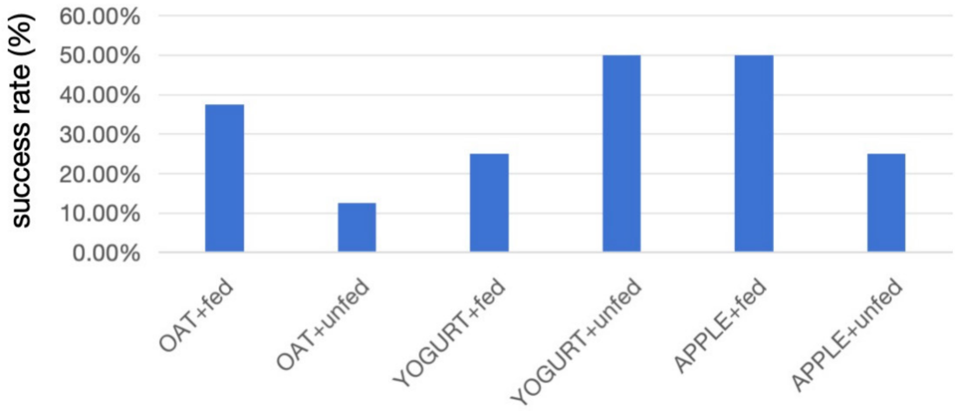


Figure 7 The success rate of the ballistic logic gates

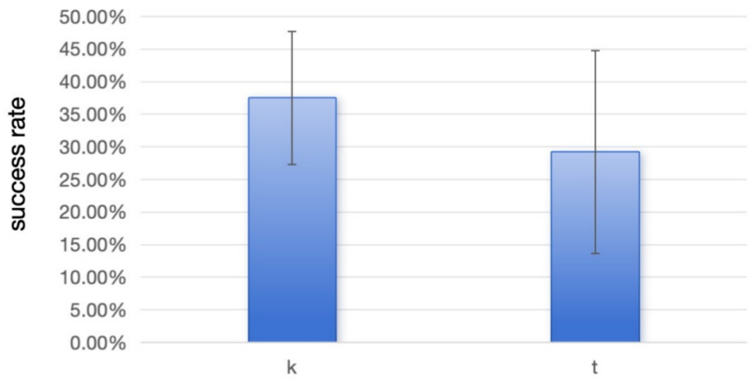
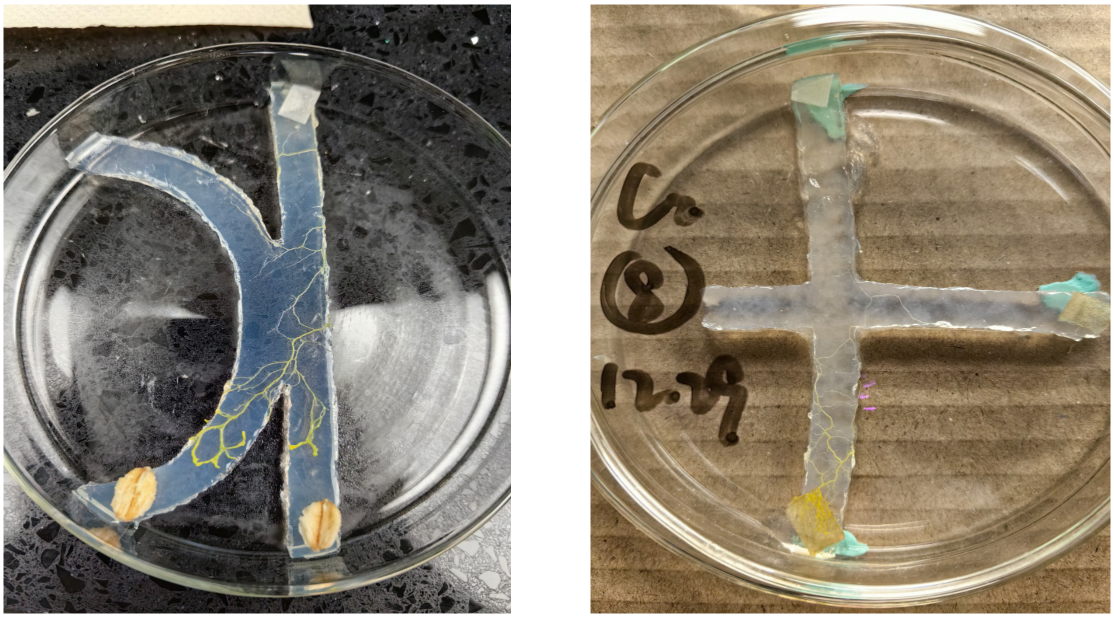


Figure 8 The average success rate for two maze shapes



(a) **(b)**

Figure 9 Experimental examples of Physarum polycephalum logic gate results, with (a) a "fail" in "k" maze, and (b) a "success" in "t" maze

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5. Discussion and Evaluation

From the results above, the unfed groups of slime mold took a longer time to reach the food sites, compared to the fed groups taken from a medium that has grown for some time. This is partly due to the waking time required for the unfed slime mold to grow from sclerotia, which was originally on a piece of cardboard. Acknowledging the sclerotia being time-consuming to wake during the pre-experiment beforehand and contradicting the growing findings on the sclerotia of slime mold by Krzywda (2008), the results of the exploration time are in line with the hypothesis.

These results build on existing evidence on the growing pattern and the life cycle of slime mold, further confirming the amoeboid organism can survive independently and grow immediately after splitting from the main group previously grown on the medium during pre-experiment. They also contribute to a clearer understanding that when using slime molds logic gates for programs, the waking time of slime molds can be considered. It is worth mentioning that if logic gates are connected, only the speed of reaching the initial gate will be affected by this waking time from sclerotia since the slime molds entering the following gates are grown from a particular food site chosen from the previous gate and can be defined as “fed”.

In this experiment, time was marked by time-stamped pictures with a five-minute interval. This may lead to minor errors, but the time lapse of slime mold’s growth is measured by hours, and the five-minute error is negligible. The time when the unfed groups of slime molds woke and left the card paper is not recorded, with the photographs showing the growing process of slime molds not clear enough to define criteria. Due to the lack of data on the waking time of unfed groups, the net exploration time of slime molds is unknown, and the current results are unable to confirm the growing speed difference between the fed and unfed slime molds.

However, with only eight mediums in each group tested, and with some slime molds did not reach either food sites which resulted in less exploration time being recorded, the exploration time of the slime molds had a relatively small sample size. This may cause the average time to be affected by some extreme values, with the standard deviations mostly over one hour, especially for the unfed group with oats as food site, taking 17.67 hours (±3.29). The fed group with apple as food site only had one medium with slime mold reaching a site, which may be a result by chance. The inaccuracy is also attributed to the limited observation time, only less than 24 hours. While in previously recorded experiments, including the railway maps had an observation time of over 26 hours (Tero *et al.,* 2010). In a study focusing on the overall coverage of the medium, the slime molds took on average 42 to 46 hours to grow throughout the area (Raper, 1951), though it was a larger area that required the slime molds to develop. Further research should take into account the growing time of slime mold when designing the logic gate experiment, and the waking time of slime molds when recording the results.

The study demonstrates a correlation between the food intention and the food coverage of slime mold. The fed groups were previously grown on agar gel fed by oat pieces placed randomly for the slime mold to find, and the gel with slime mold tubes near the oat pieces was taken for the experiment. Though with longer exploring time after reaching the food site, the fed groups of slime mold had on average less areas covering the food site, compared to the unfed groups. This difference in the speed of covering the output food site can be used to estimate the output speed of the logic gate once the slime mold is placed, since even when a food site is reached, the slime mold needs to completely cover the food and digest it before moving on to the next step of exploration. By considering the food coverage and the initial waking time, the overall growing speed for the unfed groups of slime mold in the ballistic logic gate is faster than the fed groups. The results might suggest that in the logic gate run by the slime mold when connected as a larger program, the speed of finishing each gate on the pathway will slow down as the slime mold comes across more food and becomes more “fed”. These results should be taken into account when determining the length of the logic gate pathway run by slime mold. This also provides a deeper understanding of setting the slime mold logic gate, with a diminishing length of the path for the slime mold to finish each gate at a similar time rather than slowing down. It is beyond the scope of this study to find whether the slime mold will come to a constant growing speed after being fed several times. Further studies are required to estimate a specific and accurate expected time for a slime mold to finish a ballistic logic gate under different food intention levels.

The results show differences in the speed of covering different types of food. The oats were the fastest to be covered, with an average of 2.75 coverage index, while sliced apples were the slowest. As mentioned, the slime molds were fed by oats in the fed groups before participating in the experiment. Therefore, the oats may quickly be covered due to the slime molds being familiar with this kind of food. Despite this factor, the speed of covering the food site also differs between yogurt and apple, which may be a result of the slime mold preferring yogurt rather than apple, or the soft texture of yogurt being easier to climb on for slime molds than apple flesh. While previous studies on slime molds focus on the growing speed of agar gel when fed different types of food, and the food preference of slime molds (Rawshan and Davidoff, 2023), these results demonstrate that the slime mold grows at different speeds at different speeds on different food. With a combination of the two parts of the slime mold's behavior on the ballistic logic gate, the growing speed on agar gel and food, the ballistic logic gate can be set at different speeds by changing the food type at the output sites, and the fed/unfed status of slime mold at the input sites. In the existing literature, the food-related experiment also includes the different growing speeds of slime mold when fed different food, covering oats, barley, and quinoa (Rawshan and Davidoff, 2023).

While the type of food does not necessarily impact the slime mold's decision-making, its overall growth is affected by various factors as it balances accuracy and speed through the transportation of minerals within its cell (Latty and Beekman, 2011). These pieces of evidence show that there may be more to consider when choosing food acting as a food site in the ballistic logic gate. The generalisability of the results is limited by the sample size, and the confounding variables may have affected the slime mold's food adaptability, including the previously fed type of food (oats), and the texture of food (main difference between yogurt and the other two counterparts). Avenues for future research include the effectiveness of the different types of food provided during the fed/unfed setting stage and the difference in slime mold's growing speed on different textures.

Furthermore, in the results from the experiment, the average success rate for all groups is relatively low, even compared to previous research, which resulted in 59% for the "t" shaped maze, and 69% for the "k" shaped maze (Adamatzky, 2010). The average success rates resulting from the two maze shape settings are still in line with the existing data, with the "k" shaped maze being easier to succeed (37.5%) for slime molds compared to the "t" shaped maze (29.2%). The success rate results contradicted the hypothesis, which expected rates closer to those achieved in similar experiments by predecessors. This can be a result of several reasons. Firstly, the overall number of observed slime molds is relatively low, so the success rate can be affected by chance. Secondly, the slime molds may be distracted by the remaining agar gel in the medium that had not been completely cleared after shaping the gel with the 3D-printed model maze. Thirdly, the different ingredients of the agar gel may have effects on slime mold behaviour. In the experiment carried out by Adamatzky in 2010, the agar gel used was 2% corn meal agar, while in this study, the agar gel used only contained agar and no other nutrients. In previous study, the behavioral characteristic of slime mold in determining a certain direction when exploring was suggested (Adamatzky, 2010), which is the main reason the ballistic logic gate run by slime mold can work. With the low success rate from the experiment above, however, the results do not fit with the directing theory, and the feasibility of this type of logic gate is still left to be discussed.

Apart from the low success rate from choosing the unexpected output site, some slime molds growing in both directions are a phenomenon worth mentioning, which is also against the existing theory. Regarding the method of previous research, which included 28 trials when discovering the straight-through growing behaviour of the slime mold, in 21 trials the slime mold chose to travel in the expected direction (Adamatzky, 2010). This further suggests that the low success rate in this study might be due to the small sample size. Future studies are needed to investigate the success rate with larger sample numbers, and the direction-determining behaviour of slime molds on clear agar gel mazes, under more controlled environmental factors that may affect the growth choices of slime mold.

6. Conclusion

The major question of this research is aimed at the effecting factors of slime mold's growing speed and route choices in a ballistic logic gate. Experiments on slime molds in the same logic gate maze settings of different statuses and food at the ending output site were carried out.

Six groups of slime mold were grown over a period of around 20 hours, each containing 8 mediums, with the same numbers of two different maze shapes. All recordings were aligned using a time-lapse camera, showing a series of time-stamped photographs with a constant time interval of 5 minutes. The reaching time of the slime molds touching any food existing in the maze is estimated by the photos, and the site of which the mold decided to reach is recorded. The area of the food coverage is estimated manually, depending on the overall size of the food and the percentage area the slime mold took at the end of the experiment.

Based on quantitative analysis of the collected data, this research has shown that "fed" slime molds grow faster than "unfed" slime molds on agar gel, but this speed difference is reversed on food sites. The overall growing speed of slime molds in the ballistic logic gate is faster for the "unfed" groups. Oat is the food that slime mold covers the fastest, and apple is the slowest to cover. The success rate regarding the "direction determining" growing pattern of slime mold is calculated to be lower than expected.

Though the sample size limits the generalisability of the

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results, this research provides new insight into estimating the speed of the slime mold behaviour in the ballistic logic gate depending on the type of food given and the initial status of the mold. To better understand the implications of these results, future studies could address the other factors affecting the success rate and the net growing speed of the unfed slime molds. Further research could also elaborate on the slime mold "direction determining" behaviour to confirm the feasibility of the logic gate, and to improve on the success rate for potential applications.

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