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Magnetotaxis of the *Physarum* Plasmodium and Construction of a Magnetically Controlled *Physarum* Logic Gate

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The plasmodium of *Physarum polycephalum* is a unicellular multinucleate giant amoeba. The plasmodium exhibits many types of taxes and uses these movements to adapt to its environment. In our previous study, we revealed that the plasmodium also exhibits magnetotaxis. In this study, we further investigated factors related to this magnetotaxis for use as a controlling factor in bio-computing and proposed a hypothesis on magnetotactic response of the plasmodium. Additionally, as a demonstration of magnetically controlled bio-computing, we constructed a magnetically controlled *Physarum* logic gate.

Keywords: Bio-computing, amoeba-based computing, *Physarum* plasmodium, magnetotaxis, logic gate, magnetic control

1 INTRODUCTION

The plasmodium of *Physarum polycephalum* is a unicellular multinucleate giant amoeba. At a certain stage in its life cycle a plasmodium is formed from the fusion of myriad uninucleate cells, and even after initial formation, it may fuse with other plasmodia and increase in size (Figure 1). The plasmodium is a vegetative and predatory stage of *P. polycephalum*, and thus it also increases its size by feeding, growing to a macroscopic size when

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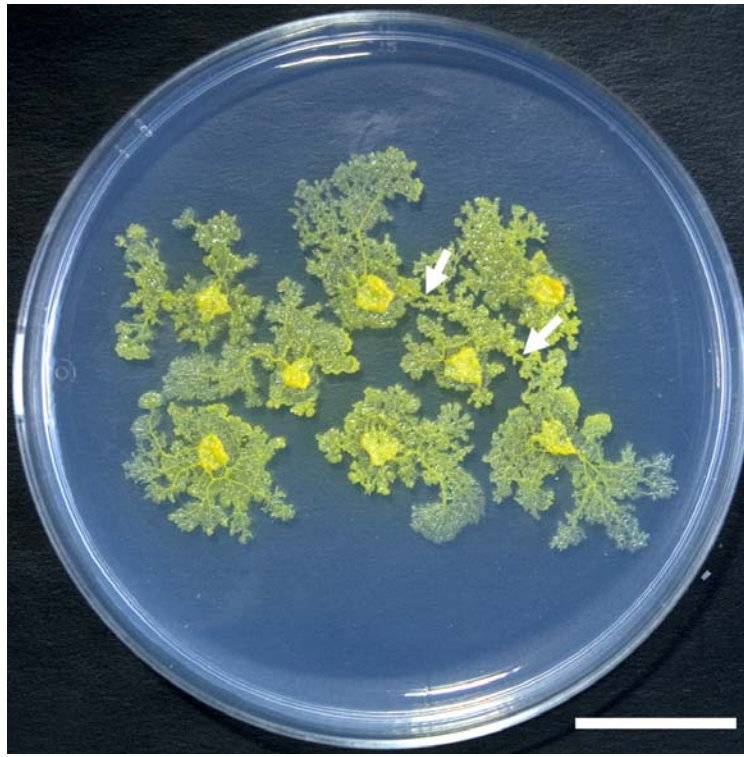

FIGURE 1
Plasmodia on 1.5 % non-nutrient agar plate in a 9-cm Petri dish. At the beginning, 8 plasmodia were inoculated on the dish. Over time, some of them made contact with each other and fused. Shared structures between fused individuals are indicated by arrows. (scale bar: 2 cm)

nutrients are sufficiently available. Though the plasmodium has a very large unicellular body, the individual parts of the cell behave cooperatively and can sense many kinds of environmental stimuli. In fact, the plasmodium exhibits many types of taxis, including chemotaxis [1,2], phototaxis [3], thermotaxis [4], geotaxis [5], and electrotaxis [6], through which adaptive behaviors are realized. These taxes also make possible the computational abilities of the plasmodium. For example, maze-solving and optimal network formation are realized through chemotaxis [1,2], and amoeboid neuro-computing using the plasmodium was realized by controlling the organism through its phototactic response [3]. Because the plasmodium has such abilities, the organism has recently attracted much attention in the field of information science, particularly in the context of bio-computing.

In our previous study, we revealed that the plasmodium also exhibits magnetotaxis, a directional movement induced by magnetic stimulus [7]. Before

Taxis	Response	Spatially controllable	Temporally controllable
Chemotaxis	Positive and negative, depending on the chemical(s)	Yes	No
Phototaxis	Negative	Yes	Yes
Thermotaxis	Positive in mild temperatures	Yes	Yes, but only slowly
Geotaxis	Positive	No	No
Electrotaxis	Negative	Yes	Yes
Magnetotaxis	Positive and negative, depending on the strength of the stimulus	Yes	Yes

TABLE 1
Features of plasmodial taxes.

our study, it had already been reported that the plasmodium changes its morphology under a strong magnetic stimulus of around 0.1 T [8]. Additionally, the fact that the plasmodium exhibits electrotaxis [6] suggested that it might also be responsive to electromagnetism. We therefore examined the behavior of the plasmodium in a magnetic field with a strength similar to the Earth's magnetic field, in contrast to the very strong magnetism used in the previous study [8]. We demonstrated that the plasmodium moves along magnetic lines toward the N-pole or S-pole, and this north/south directional movement is dependent on both its cell weight and the strength of the applied magnetic field. Such behavior is very different from those of other organisms that exhibit magnetotaxis, such as migratory birds [9] and magnetotactic bacteria [10]. Because they determine their directional orientation by the magnetic moment recorded in the magnetite structure (magnetosome) of their body, their magnetotaxis usually induces a unidirectional response. In contrast, the bidirectional nature of the magnetotaxis exhibited by the plasmodium cannot be explained from the function of a magnetosome, and is thus a newly discovered type of magnetotaxis.

The magnetotaxis of the plasmodium is an important factor in its application as a bio-computer because changing the strength of a magnetic stimulus enables spatio-temporal and behavioral control of the organism. The features of plasmodial taxes are summarized in Table 1. The use of phototaxis, electrotaxis, and magnetotaxis enables both spatial and temporal control of the input to the plasmodium. Of these, electrotaxis is the least promising because an electric current induces electrochemical reactions in biological media that are usually harmful. Phototaxis is a better candidate as a controller of plasmodial computing, and in fact, *Physarum* neuro-computing has already been

realized through the use of light stimulus [3]. Control by magnetotaxis has further merit because the response of the plasmodium can be controlled by changing the strength of the stimulus, thereby enabling more flexible control.

In this study, we further investigated the factors related to the magnetotactic response of the plasmodium that are necessary to establish magnetically controlled *Physarum* computing. In addition, as a demonstration of a *Physarum* computing device, we constructed a magnetically controlled *Physarum* logic gate.

2 MATERIALS AND METHODS

2.1 Plasmodium culture

All of the plasmodia used in this study were cultured using the method developed by Camp [11]. In a 30 cm $\times$ 20 cm plastic box, 9-cm glass Petri dishes were tightly arranged to minimize the space between them. Wet paper towels were placed on the dishes, and the space below the towels was filled with water to maintain the moisture in the box and on towel surface. Plasmodia were cultured on the paper towels, while the box was kept in the dark and the room temperature was maintained at 23°C. The plasmodia were fed daily with oatmeal flakes and within 24 h before the start of the experiments. The fresh locomotive fronts of the plasmodia were scraped, weighed, and used for the experiments.

2.2 Experimental setup

The experimental setup is shown in Figure 2a. All experiments were performed in a hand-made cubic chamber assembled from 60-cm-long L-type steel angles covered in plastic sheeting to maintain the internal moisture. Before the experiment, water was sprayed throughout the inside of the

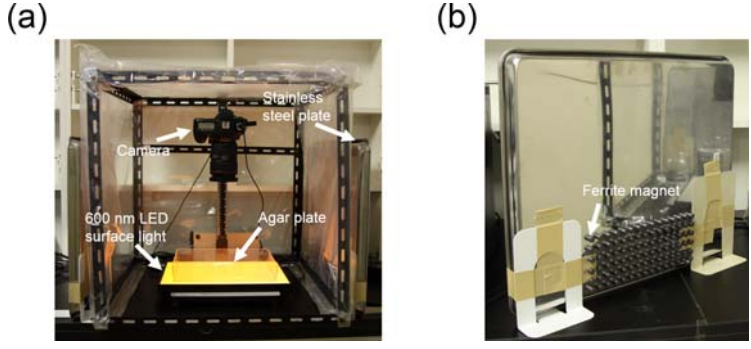

FIGURE 2
(a) Experimental setup. (b) Stainless steel plate with ferrite magnets attached.

chamber to supply moisture, and a 1-liter beaker of water was placed in the chamber to maintain the moisture levels. During the experiments, the chamber was kept closed, and the humidity in the chamber was maintained at greater than 80 %.

A digital camera (EOS 5D Mark II, Canon, Japan) attached to a copy stand (King 731265, Asanuma & Co., Ltd., Japan) was placed in the center of the chamber. A surface light source consisting of 600 nm light emitting diodes (custom-made item, Aitec System Co., Ltd., Japan) was placed on the lower surface of the copy stand, and in the experiments, a 30 cm $\times$ 20 cm plastic box containing a 1.5 % non-nutrient agar plate was placed on the surface light source. The light from the 600-nm light emitting diodes was used because it is outside the range of light that induces phototaxis in the plasmodium [12]. The agar plate was inoculated with plasmodia and photographed using the digital camera from directly above.

Ferrite magnets were attached in a 6 $\times$ 15 grid pattern to two stainless steel plates (4-5309-14, As One Corp., Japan) (Figure 2b). The plates were arranged such that the magnets of one faced the N-pole outward and the magnets of the other faced the S-pole outward. The plates were placed, outside or inside the chamber to provide a homogeneous magnetic field and to apply magnetism to the plasmodium. The strength of the magnetic field was confirmed using a hand-held gaussmeter (Model 410, Lake Shore Cryotronics, Inc., USA).

All the experiments were performed in the dark at a temperature of 23°C.

2.3 Observation of 2-dimensional plasmodial movement in a magnetic field

The center of the agar plate in the experimental setup described in section 2.2 was inoculated with a 5, 10 or 15 mg section of the plasmodium (Figure 3a). A magnetic field of 0.1 or 0.3 mT was applied to the experimental field. These magnetic field strengths included the influence of the Earth's magnetic field. The plasmodium was photographed at 5-min intervals for 24 h. From the resulting images, the position of the plasmodium at each time step was detected based on its center of mass by using image processing software (ImageJ, National Institutes of Health, USA) [13]. The motion vector of the plasmodial movement at each time step was also calculated based on these data. The bias in the movement direction of the plasmodium was statistically analyzed using the Rayleigh test and Kuiper's test.

2.4 Observation of 1-dimensional plasmodial movement in a magnetic field

The experimental setup described in section 2.2 was used. One-dimensional paths of agar medium were prepared by cutting ditches into the agar plate

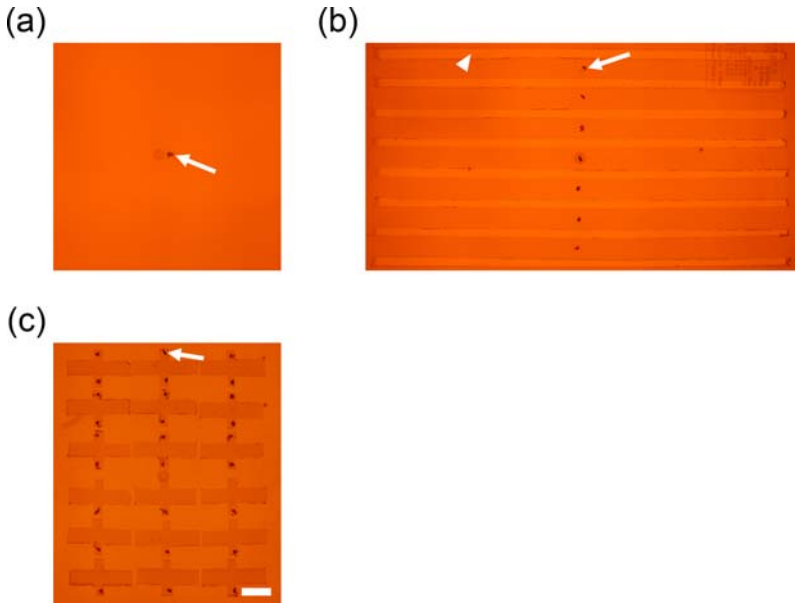

FIGURE 3
Experimental setups used for (a) observation of the plasmodium in a 2-dimensional space under the application of the magnetic field, (b) observation of the plasmodium in a 1-dimensional path under the application of the magnetic field, (c) *Physarum* logic gate. The right side of the images corresponds to the northern direction. The arrows indicate the plasmodium immediately after inoculation, and the arrowhead indicate the ditch in the agar plate surface. (scale bar: 2 cm)

surface (Figure 3b). The width of the ditches was 0.5 cm and that of the paths between the ditches was 1.5 cm. Plasmodia of the same weight were inoculated onto the centers of the paths. The weights of the plasmodia ranged from 5 to 80 mg, and the strengths of the magnetic field applied were 0.1, 0.2, and 1.0 mT. In this experiment too, the magnetic field strengths included the influence of the Earth's magnetic field. For the application of the 1.0 mT magnetic field, neodymium magnets were used instead of ferrite magnets. The magnetic fields were applied along the direction of the paths. The plasmodia were photographed at 5-min intervals for 24 h, and the directional movement, that is, whether the plasmodia moved toward the N-pole or S-pole, was observed.

2.5 Construction of a magnetically controlled *Physarum* logic gate

The experimental setup described in section 2.2 was used; however, instead of an agar plate, the cross-shaped substrate of the *Physarum* logic gates was cut out from an agar plate and placed in the plastic box (Figure 3c). Plasmodia were inoculated onto the east and/or west side(s) of the cross-shaped

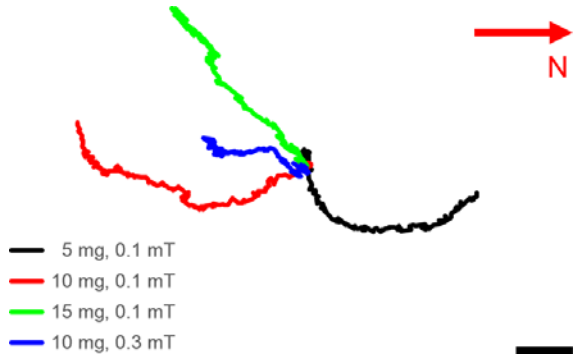

FIGURE 4
Trajectories of the center of mass of the plasmodia under application of a magnetic field. The right side of the image corresponds to the northern direction. (scale bar: 1 cm)

substrate as inputs for the logic gates. The plasmodia were photographed at 5-min intervals for 24 h, and the directional movement toward the N-pole or S-pole, were observed as outputs. The original RGB images were converted to 8-bit grey scale and enhanced using ImageJ [13].

3 RESULTS

3.1 Result of 2-dimensional experiment

First, we observed the movement of the plasmodium under application of a magnetic field. The plasmodium was inoculated onto a flat agar surface, where the organism could move freely in two dimensions. In our previous study, we had already observed that the behavior of the plasmodium was dependent on its weight and the strength of the magnetic field applied [7]; therefore, we performed this experiment by varying the weight of the plasmodia and the strength of the magnetic field. Figure 4 shows the trajectories of the plasmodia in the experimental field, and Figure 5 shows the frequency distribution of those trajectories. As shown in these figures, plasmodial movement was biased toward a northern or southern direction, along the magnetic lines. For the samples shown in Figure 5, mean vectors were calculated as (a) 349.4°, (b) 165.7°, (c) 129.2°, and (d) 163.0°. Their directional bias was statistically confirmed using the Rayleigh test ($p < .025$ for (a), $p < .001$ for (b-d)) and Kuiper's test ($p < .025$ for (a), $p < .01$ for (b-d)).

3.2 Result of 1-dimensional experiment

To further investigate factors related to plasmodial magnetotaxis, namely, cell weight and magnetic field strength, we performed an experiment using 1-dimensional paths. In so doing, we increased the number of samples we

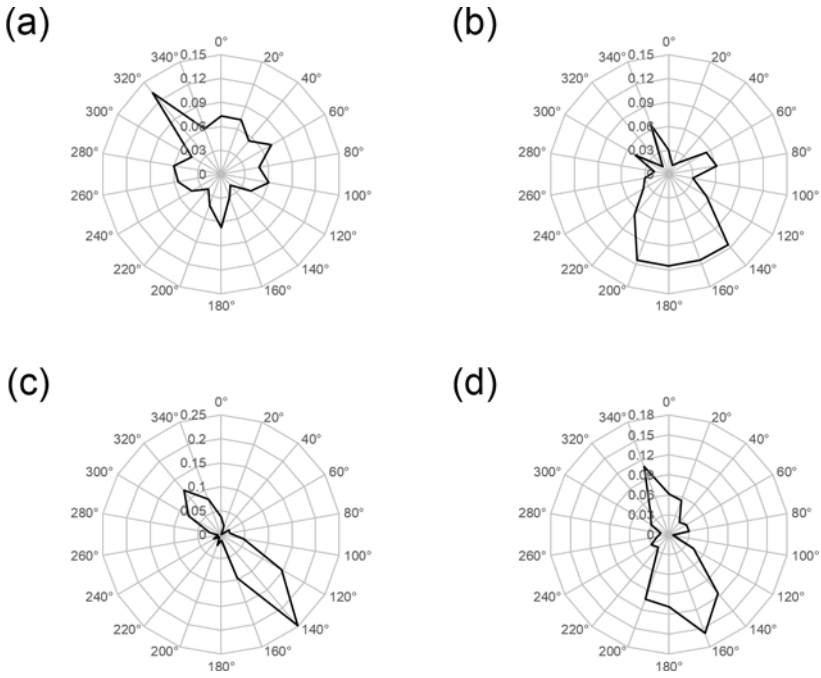

FIGURE 5
Frequency distribution of the directions of the trajectories shown in Figure 4 corresponding to the following plasmodial weights and magnetic field strengths: (a) 5 mg, 0.1 mT, (b) 10 mg, 0.1 mT, (c) 15 mg, 0.1 mT, (d) 10 mg, 0.3 mT. The directions 0° and 180° correspond to north and south, respectively. The distribution of (a) is biased toward the north; the others are biased toward the south.

could simultaneously observe because ditches prevented the plasmodia from interfering with each other and allowed us to observe whether the plasmodia moved toward the north or south.

First, we reconfirmed the dependency of the magnetotaxis on cell weight (Table 2). As expected, the directional movement of the plasmodium differed according to cell weight. We performed another experiment using different cell weights and magnetic field strengths (Table 3). The data in Table 3 clearly indicates that the directional movement of the plasmodia differed according to both cell weight and magnetic field strength. Table 4 integrates the results from Tables 2 and 3, and shows the relationship of directional movement with the product of cell weight and magnetic field strength. The results of this study indicate that the magnetotactic response of the plasmodium is determined by the product of cell weight and magnetic field strength, and that the north/south directional movement reverses when the value of the product quadruples.

Cell weight (mg)	Direction
5	N: 5 S: 0 ($p < 0.05$)*
7.5	N: 5 S: 0 ($p < 0.05$)*
9	N: 0 S: 5 ($p < 0.05$)*
10	N: 1 S: 4 ($p = 0.156$)
20	N: 0 S: 5 ($p < 0.05$)*

TABLE 2
Results from the experiment using 1-dimensional paths and various cell weights. N and S indicate the number of samples that moved toward the north and south, respectively. Conditions where N and S are dominant are colored red green, respectively. The *p*-values are from a binomial test; statistical significance is indicated by*.

3.3 Operation of a magnetically controlled *Physarum* logic gate

As an example of a magnetically controlled *Physarum* computing implementation, we constructed a *Physarum* logic gate (AND-gate) that operates based on the magnetotaxis of the plasmodium. A diagram of a *Physarum* AND-gate is shown in Figure 6. The AND-gate is constructed by using 5 mg plasmodial sections as inputs and by applying a 0.1 mT magnetic field. In this logic gate, the presence of the plasmodium is regarded as the presence of an input and output. When the input is (0, 0), nothing happens and consequently the output is 0. When the input is (0, 1) or (1, 0), the 5 mg plasmodial section proceeds to the center of the gate. Then, under the application of a 0.1 mT magnetic field, the 5 mg plasmodium moves toward the north (Table 4) and thus the output is 0. When the input is (1, 1), the two plasmodial sections meet at the center of the gate and fuse, resulting in a 10 mg plasmodium that moves toward the south (Table 4) and the output is 1.

		Magnetic field strength (mT)		
		0.1	0.2	1.0
Cell weight (mg)	5	N: 10 S: 0 ($p < 0.001$)*	N: 0 S: 5 ($p < 0.05$)*	N: 3 S: 1 ($p = 0.25$)
	20	N: 0 S: 10 ($p < 0.001$)*	N: 5 S: 0 ($p < 0.05$)*	N: 1 S: 3 ($p = 0.25$)
	80	N: 3 S: 1 ($p = 0.25$)	N: 0 S: 4 ($p = 0.0625$)	N: 3 S: 1 ($p = 0.25$)

TABLE 3
Results from the experiment using 1-dimensional paths and various cell weights and magnetic field strengths. N and S indicate the number of samples moved to north and south, respectively. The total number of the samples tested in each condition is a sum of them. Conditions where N and S are dominant are colored red and green, respectively. The *p*-values are from a binomial test; statistical significance is indicated by*.

Cell weight (mg)	Magnetic field strength (mT)	Product of cell weight and magnetic field strength (mg·mT)	Direction
5	0.1	0.5	N
7.5	0.1	0.75	N
9	0.1	0.9	S
10	0.1	1	S
5	0.2	1	S
20	0.1	2	S
20	0.2	4	N
5	1	5	N
80	0.1	8	N
80	0.2	16	S
20	1	20	S
80	1	80	N

TABLE 4
Integrated results of Tables 2 and 3, showing the relationship between the north/south directional movement and the product of cell weight and magnetic field strength. Conditions where N and S are dominant are colored red and green, respectively.

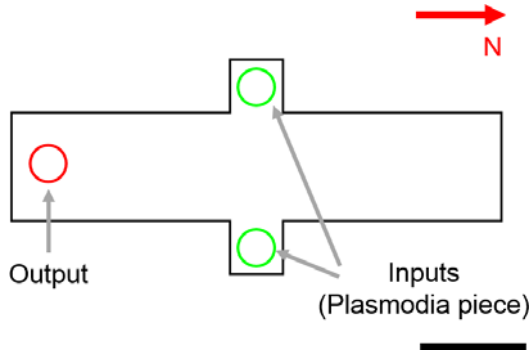

FIGURE 6
Diagram of *Physarum* AND-gate. As inputs for the gate, plasmodia were inoculated on the east and/or west side(s) of the cross-shaped gate substrate, which were perpendicular to the magnetic field line. If the plasmodium reaches the southern end, the value of the output is regarded as 1. (scale bar: 1 cm)

FIGURE 7
Operation of the *Physarum* AND-gate with input (1, 0). (a) Immediately after inoculation. A 5 mg plasmodial section is indicated by the arrow. (b) 100 min after inoculation. The plasmodium proceeded to the center of the substrate. (c) 160 min after inoculation. The plasmodium moved toward the north, which is the opposite side of the output position. (scale bar: 1 cm)

FIGURE 8
Operation of the *Physarum* AND-gate with input (1, 1). (a) Immediately after inoculation. The two 5 mg plasmodial sections are indicated by arrows. (b) 140 min after inoculation. The plasmodia proceeded to the center of the substrate and fused. (c) 200 min after inoculation. The plasmodia moved toward the south and reached the output position. (scale bar: 1 cm)

We experimentally constructed and operated the *Physarum* AND-gate described above. Example results with inputs of (1, 0) and (1, 1) are shown in Figures 7 and 8, respectively. Both gates operated as expected; the former's output was 0 and the latter's output was 1.

4 DISCUSSION

In this study, we investigated the factors related to the magnetotaxis exhibited by the *Physarum* plasmodium, demonstrating its potential application in biocomputing by constructing a magnetically controlled *Physarum* logic gate. In its magnetotactic response, the plasmodium showed a tendency to move in the direction of the magnetic lines. The results of this study indicate that the north/south directional movement is determined by the product of cell weight and magnetic field strength, reversing when the value of the product quadruples.

We constructed a *Physarum* AND-gate by manipulating the abovementioned factors related to plasmodial magnetotaxis and demonstrated examples of its successful functioning. However, we admit that its development is still in a preliminary stage; it does not always perform as intended and thus requires further improvements to achieve greater stability. For example, when two plasmodia are inoculated as input (1, 1), they sometimes fail to encounter each other and fuse, and the intended output is not obtained. In another case with input (1, 1), if the fusion of the two plasmodia occurs too late, the individual plasmodia begin to exhibit a magnetotactic response based on their cell weight and the intended output is not obtained. We suppose that such problems can be solved by improving the design of the logic gate, especially by decreasing its size so that multiple plasmodia can encounter one another faster. Faster operation resulting from downsizing the gate is possible if the "quadruple rule" described in the last part of section 3.2 is true, because the same logic gate design can be made at a smaller scale. We will investigate this point further in a future study.

Physarum logic gate has already been constructed in the previous study, based on plasmodial chemotaxis [14]. However, the magnetically controlled *Physarum* logic gate in this study has some advantages, as discussed below. Hereinafter, the logic gate in the previous study is referred to as the chemotactic logic gate, and that of the present study is referred to as the magnetotactic logic gate. First, the structure of the magnetotactic logic gate is simpler than that of the chemotactic logic gate. The magnetotactic AND-gate is realized with a simple cross-shaped structure, while the chemotactic AND-gate requires a 3-dimesional structure. Second, the magnetotactic logic gate can perform several types of operation by using the single cross-shaped structure. For example, we can construct an OR-gate by using the structure illustrated in Figure 6 with 5 mg plasmodia as inputs and changing the strength of the magnetic field from 0.1 mT to 0.2 mT. Provided that the quadruple rule is true, 5 and 10 mg plasmodia move toward the south under the application of a 0.2 mT magnetic field and the inputs (0, 1), (1, 0), and (1, 1) will yield an output of 1. By the same principle, an XOR-gate becomes possible with

a 0.4 mT magnetic field. In contrast, the structure of the chemotactic logic-gate is operation-specific; different structures are needed for different logical operations. Finally, the magnetotactic logic gate allows for a sequential operation. Because the structure of the magnetotactic logic gate is operation-independent, there is no difference in operation time derived from the operation type. Therefore, output from the gates in the first stage (e.g., outputs from an AND-gate and an OR-gate) can be used sequentially as inputs in the second stage. This is difficult for the chemotactic logic gate to achieve because of its operation-specific structure.

The mechanism of plasmodial magnetotaxis remains unclear; however, its elucidation is beyond the scope of this study. If the quadruple rule is indeed true, it may offer a clue. The weight of the plasmodium is proportional to the area covered by its body because the plasmodium is essentially 2-dimensional and its area is the square of its length. Therefore, the quadruple rule may be interpreted such that the north/south directional movement is determined by the product of the cell length and the magnetic field strength, which reverses when the value of the product doubles. We further hypothesize that the electromagnetic interaction between the magnetic field and cytoplasmic streaming (the intracellular flow that includes charged ions) in the longitudinal direction of the cell plays a key role in determining the magnetotactic direction, but it remains a challenge to predict how such interaction leads to movement toward the north instead of the south or vice versa. Further investigation into the quadruple rule may be the first step toward understanding the mechanism of *Physarum* magnetotaxis and its application in bio-computing.

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