Anticipatory Aspect in the Cell Motility of *Physarum* Plasmodium

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Abstract
We used the plasmodium of *Physarum polycephalum* to study adaptive, exploratory and anticipatory behavior of cellular system. Following our previous study, we investigated how the tubular structure of the plasmodium affects the cell motility. From a series of experimental results, it was indicated that the cell motility and consequent morphogenesis is dependent on the locomotive history of the plasmodium. We thus propose the presence of memory reservoir in the cellular organism, and discuss how such aspect is related to computing anticipatory systems.
Keywords: *Physarum polycephalum*, bio-computing, morphogenesis, cell motility, cellular anticipatory system

1 Introduction

The plasmodium of *Physarum polycephalum* is a unicellular and multinuclear amoeboid organism, which grows up to centimeter- or meter-scale if cultured in an appropriate condition. In spite of the massive volume of the cell body, each local part of the organism does not behave independently and the whole cell body seems to have a global decision. Such an aspect allows the amoeba to show adaptive and intelligent behavior in its environment, and so far it has been demonstrated that we can use the organism in the computations for maze-solution (Nakagaki et al., 2000a, 2001; Nakagaki 2001), minimum spanning tree and Steiner minimum tree (Nakagaki et al., 2003, 2004), and Voronoi diagram (Adamatzky et al., 2008; Shirakawa et al., 2009). The systems for neuro-computing (Aono and Hara, 2008), logical gate construction (Tsuda et al., 2004) and robot control (Tsuda et al., 2007) have also been implemented using the plasmodium. The organism is thus widely attracting a lot of attention in terms of bio-computing and systems science, beyond its original importance in biology.

The *Physarum* plasmodium roughly consists of two parts; sheet-like structure of locomotive front (pseudopodium) and tubular structure at rear part (Fig 1). The sheet-like pseudopodia rigorously crawls on plane surfaces and stretches itself to reach its food sources. All the parts of cell body, however, remain connected by cellular tubes so as to maintain integrated unity as a single cell. Since the plasmodium in this process saves the cell volume required for constructing tubes as small as possible, it is able to find a solution for a maze (Nakagaki et al., 2000a, 2001; Nakagaki 2001) or an efficient network connecting multiple food sources (Nakagaki et al., 2003, 2004).

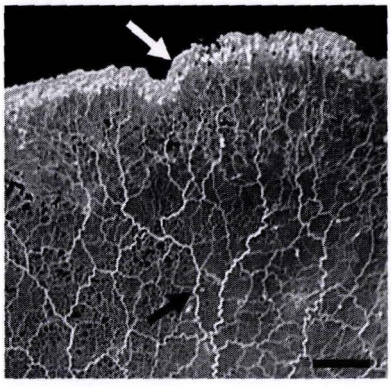

Figure 1: The plasmodium of *Physarum polycephalum* spreading on 1.5 % agar gel. The white arrow in the figure indicates the sheet-like locomotive front, and the black arrow indicates the tube in rear part. (Scale Bar: 1 cm)

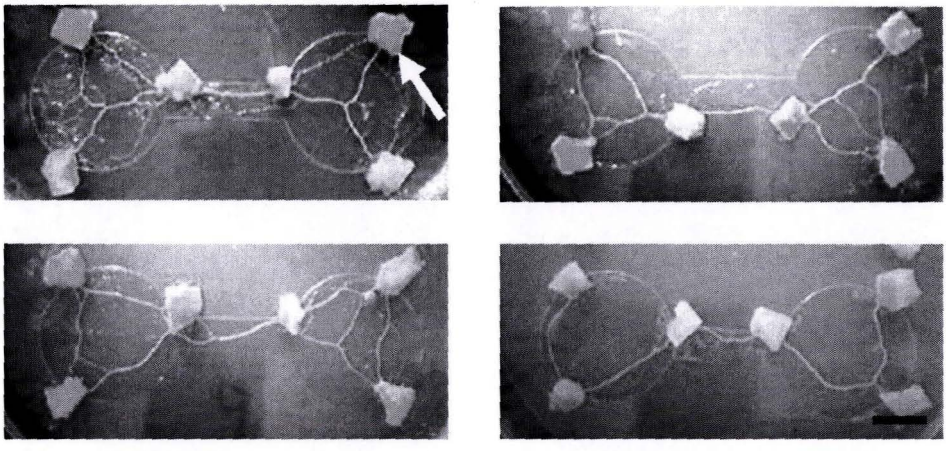

Figure 2: Examples of plasmodium networks that showed homologous morphology. In each figure, two sub-networks in the circular fields showed homology, connecting the food sources (white arrow). Note that in each figure the sub-networks consist of a single cell. For further details, refer to Shirakawa and Gunji, 2008. (Scale Bar: 1 cm)

In our previous study, we compared the local morphologies of sub-networks of the plasmodium to study local intracellular interaction that would lead to global decision of the cell. We found that the local networks of the plasmodium have a tendency to form homologous morphology (Shirakawa and Gunji, 2007 and Fig. 2). This result indicated a presence of some mechanism that transmitted the local state or locally received environmental information of the cell to the other part. Additionally, thinking of globally cooperative behavior of the plasmodium, it is possible to assume the presence of information integrating mechanism within the cellular organism.

In this study, to investigate the mechanism that causes the emergence of morphological homology described above, we studied how presence of tube affects the cell motility and morphogenesis (tubulogenesis). A series of experiments revealed that presence of tube give rise to increase in complexity of plasmodium morphology, and in the velocity of cell motility. Furthermore, we also found that tubular trail formed after cellular locomotion affected the directionality of later locomotion and morphogenesis. In other words, locomotive history of *Physarum* plasmodium affected the future cell motility and morphology. We propose that the tubes work as a reservoir for "memory", and that the *Physarum* plasmodium is a good example of cellular anticipatory system.

2 Materials and Methods

2.1 Culture of *Physarum* Plasmodia

The plasmodia of *Physarum polycephalum* were cultured using the method of Camp (Camp, 1936). Wet paper towels were laid out on glass Petri dishes in a plastic box. The space below the towels was filled with water to supply moisture. The plasmodia were cultured at 24 °C on the wet paper towels, and fed daily with oatmeal. The sheet-like locomotive front of the plasmodium in the culture was scraped out and weighed, then supplied for the experiments.

2.2 Preparation of Artificial Plasmodium Tubes

Into a 200 ml paper cup, 180 ml of 1.5 % melted agar was poured. Then 2 stainless pipes 1 mm in diameter were let penetrate the agar solution to the bottom face of the paper cup, and the pipes were fixed. After the gelation of the agar solution, the pipes were picked out and in such a way mass of 1.5 % agar with two 1 mm holes was prepared. The bottom face of the paper cup was cut out to make up the holes open-ended, and 400 mg of *Physarum* plasmodium was inoculated on the top face of the agar mass. By positive geotaxis, the inoculated plasmodium went downward to the bottom, forming cellular tubes in the agar holes. The plasmodium tubes surrounded with agar gel were carved out from the agar mass, and used in the experiments.

All the processes described above are schematized in Figure 3.

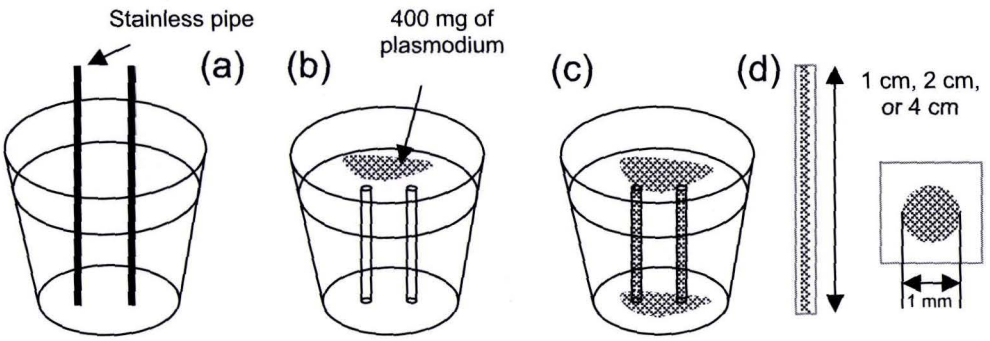

Figure 3: A schema for the construction of artificial plasmodium tubes. (a) Melted agar is poured to a paper cup, and two stainless pipes are set in the gel solution. (b) After the solidification of the agar, the stainless pipes are picked up leaving 2 holes in the agar mass. Then, the bottom surface of the paper cup is cut out and 400 mg of plasmodium is inoculated on the top surface. (c) The plasmodium goes down in the holes, forming tubes inside. (d) Side view and top view of a plasmodium tube carved out from the agar mass in (c). In the top view, the thickness of agar gel surrounding the plasmodium tube is less than 1 mm.

2.3 Experimental Setup

All the experiments in this study were performed on 1.5 % agar plate in a 9 cm Petri dish at 24 °C. All of the images were taken using a digital camera EOS kiss ditital-X (CANON, Japan). Yellow light 600 nm in wavelength was used as a light source, so as not to invoke the photo avoidance of the plasmodium. The images were enhanced and processed using Adobe Photoshop CS3 (Adobe Systems Inc, CA, USA) and ImageJ (Rasband, 1997-2008). In experiment II and III, the experimental fields were prepared by fabricating 0.1 mm-thick plastic films and placing the processed film on 1.5 % agar plate.

2.3.1 Setup for Experiment I

Twenty mg of the plasmodium was inoculated at the center of an agar plate. Then 2 cm or 4 cm artificial plasmodium tube was transplanted to the 20 mg mass. Control experiment was performed without the tube. The samples were set still for 2 hours, and then photographed.

2.3.2 Setup for Experiment II

A 70 mm linear path was prepared on an agar plate as an experimental field (Fig. 6). Fifteen mg of the plasmodium was inoculated at the center of the path. Then 1 cm, 2 cm or 4 cm artificial plasmodium tube was transplanted to the 15 mg mass. Control experiment was performed without the tube. The sample was photographed at 5 min. intervals for 12 hours.

2.3.3 Setup for Experiment III

A linear or L-shaped 40 mm path was prepared on an agar plate as an experimental field, keeping the opposite side of the plate without any obstacles (Figs. 8, 9). Forty mg of the plasmodium was inoculated at the dead end of the path. The sample was set still until the plasmodium reached the open end and entered the obstacle-free plane. The sample was set still for additional 4 hours, and then photographed. The distribution of cytoplasm on the obstacle-free plane was calculated according to Beer-Lambert law, and the position of the center of mass was detected.

3 Results

3.1 Experiment I. Observation of 2 Dimensional Cell Motility of the Plasmodium Transplanted with Artificial Plasmodium Tube

To test how the presence of tube affects on plasmodium cell motility and consequent morphogenesis, we transplanted artificially constructed plasmodium tube to plasmodium mass, in experiment I and experiment II. We studied 2 dimensional motility in the former experiment, and 1 dimensional motility in the latter.
In experiment I, transplantation of tube caused visibly clear alteration in morphology of the sheet-like structure: there was a drastic change in heterogeneity of the morphology (Fig. 4).

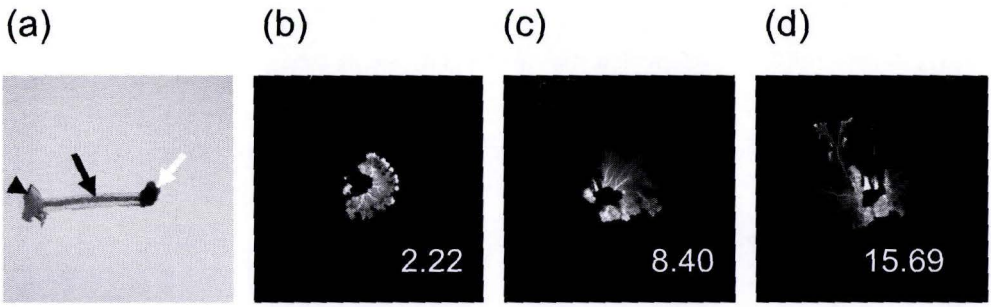

Figure 4: The set up for experiment I and the results. (a) The set up for experiment I. Twenty mg of the plasmodium mass (white arrow) was transplanted with artificially constructed plasmodium tube (black arrow). In this figure, 2 cm tube is used. The open end of the plasmodium tube was sealed with vaseline (arrowhead). (b)-(d) The results of experiment I. The images were taken 120 min. after the inoculation and transplantation, and the images subtracted with the images at 0 min. to enhance the morphology of the plasmodium. Thus in the images the transplanted tubes are not visible. (b) With no tube. (c) With 2 cm tube. (d) With 4 cm tube. Compared with (b), more complex morphlogy is observed in (c), and complexity further increases in (d). The numbers in (b)-(d) indicate the complexity index of each sample. (Scale Bar: 1 cm)

We thus quantified the degree of complexity in the morphology of the plasmodium, using a dimensionless measure $L^2/4\pi S$. Here L indicates perimeter, and S indicates area

that plasmodium is covering. We designated this measure "complexity index". With a round shape the complexity index indicates 1, and according to the increase of complexity and deviation from round shape, the complexity index increases. We tested 5 samples in each experimental condition, and the average of complexity index was calculated (Fig. 5). The complexity index linearly increased with the length of artificial tube transplanted.

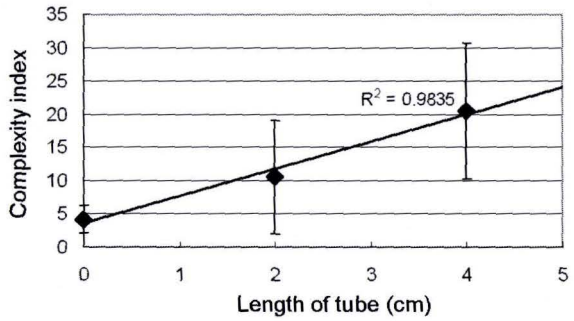

Figure 5: The complexity index increased linearly with the length of the transplanted tube. "0 cm" indicates the results of control experiment. Significant difference in average value of complexity index among each condition was detected by ANOVA ($P < 0.05$).

3.2 Experiment II. Observation of 1 Dimensional Cell Motility of the Plasmodium Transplanted with Artificial Plasmodium Tube

In the experiment II, we tested the effect of tube transplantation on 1 dimensional cell motility of the plasmodium. The experimental system is indicated in Figure 6. We tested 8 or 9 samples in each experimental condition. The velocity of cell motility linearly increased with the length of artificial tube transplanted.

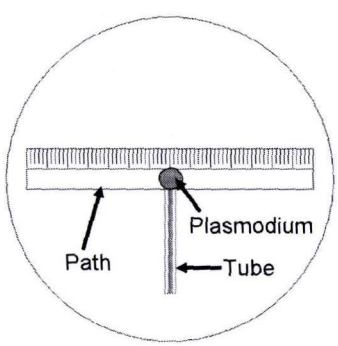

Figure 6: The set up for experiment II. The plasmodium was inoculated on the center of the path, and tube was transplanted. The plasmodium moved to the right and left, and the velocity of the locomotion was measured. The velocities to the right and to the left were averaged and displayed in Figure 7.

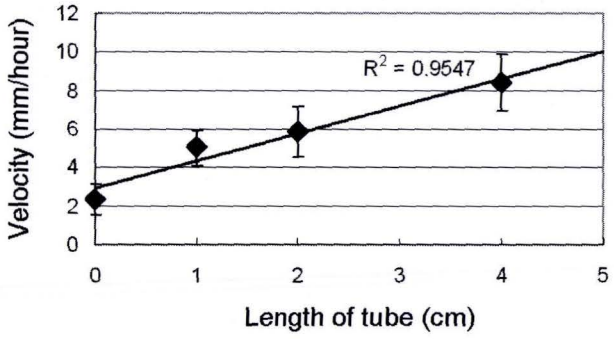

Figure 7: The velocity of cell motility increased linearly with the length of the transplanted tube. "0 cm" indicates the results of control experiment. Significant difference in average velocity among each condition was detected by ANOVA ($P < 0.01$).

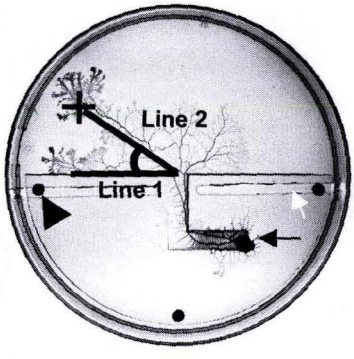

Figure 8: An example of a result from experiment III. Forty mg of the plasmodium was inoculated on the point indicated by the black arrow. The image was taken 4 hours after the plasmodium reached the open end of the path. The markers (arrowhead) were just used to adjust the position of the plastic film. The ditches in the agar plate (white arrow) were scraped out to avoid possible effect of electric potential generated by the cell, which can be propagated via agar plate. For the plasmodium in the obstacle-free plane, the position of the center of mass (cross) was detected. To evaluate the directionality in each sample, 2 lines, a line corresponds to the left side edge of the experimental field (Line 1) and a line connecting the center of mass position and the center of the open end of the path (Line 2) were drawn. Then the angle between the two lines was measured as indicated in the figure, and used as a criterion to estimate the directionality.

3.3 Experiment III. Test for Effect of Locomotive History on Cell Motility

In experiment II, the plasmodium showed increased velocity of cell motility with the transplantation, and at the same time the plasmodium displayed increased degree of directionality. In other words, the plasmodium without tube transplantation was apt to move right and left simultaneously, but the plasmodium with tube tended to show unidirectional movement. Also in experiment I, the increase in morphological complexity can be explained that this is because increased velocity and directionality promoted the heterogeneity.

We thus assumed that the arrangement of tubes generated as a consequence of cell motility plays some role in the control of direction. That is to say, we hypothesized that locomotive history has some effect on cell motility. Then we constructed an experimental system to test the hypothesis, in experiment III (Figs. 8, 9). In experiment III, the plasmodium was given three types of path (Fig. 9a-c). The motility of the plasmodium was observed after the plasmodium went through the path, and the position of the center of mass was calculated. Each pairs of 3 types of experimental results (Figs. 9a-c), significant difference in the distribution of the center of mass was detected. Namely, the distributions were dependent on the type of path, and the locomotive history. Especially in Figure 9b and 9c, the distribution was biased to the opposite side of the direction to the former half of the path..

We further investigated how such a control of cell motility is realized. As seen in the Figure 8, the cellular tubes remained in the guiding path throughout the experiment. Then we supposed the effect of the remaining tube, and scratched out the part of the tube before the locomotion of the plasmodium in the obstacle-free plane (Fig 9d).

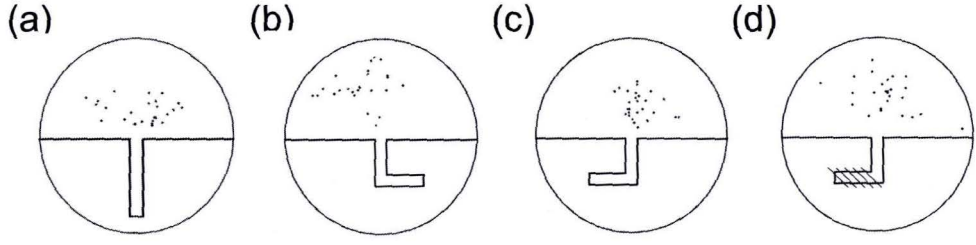

Figure 9: The set up for experiment III and the results. For plasmodium locomotion, straight (a), L-shaped (b) and reverse-L-shaped (c, d) paths were used. The plots in each figure indicate the center of mass positions, obtained from about 25 experiments each. While the plots in (a) shows relatively non-directional distribution, the plots in (b) and (c) showed clearly biased distribution to the left and right, respectively. These biases are opposite to the direction of the former half of the path. The 3 distributions in (a-c) showed significant difference ($P < 0.05$ for the pairs (a) and (b), (b) and (c), and $P < 0.01$ for the pair (b) and (c)). In (d), the tubes in shaded area was scraped out, when the plasmodium reached the open end of the path. Compared with (c), plots in (d) showed weaker bias in distribution; the pair (b) and (d) showed significant difference ($P < 0.05$), but the pair (a) and (d) displayed no detectable difference.

The distribution of plots in Figure 9d showed significant difference with that in Figure 9b, but did not show with that in Figure 9a. Therefore, compared with the distribution in Figure 9c that showed significant difference with both of that in Figure 9a and 9b, the bias in directionality in Figure 9d is weaker than that in Figure 9c. This result indicates that the arrangement of tubes as a consequence of locomotion affects the cell motility, and this is also consistent with the results from experiment I and II.

4 Discussion

To study the mechanism of cooperative behavior observed in the plasmodium of *Physarum polycephalum*, we studied how the tubular structure of the plasmodium affects the cell motility and consequent morphogenesis. Three experiments were done using the direct transplantation of tube, or the tubes formed as a trail of locomotive history of the organism. As mentioned above, the tubes are formed as a trail of locomotion, and how the motility gives an effect on the tubulogenesis has already been studied in details (Nakagaki et al., 2000b). However, so far there is no study dealing with "tube to motility" influence in details. Thus the series of experimental results from experiment I, II and III are novel ones.

Roughly speaking, the motility of the plasmodium is driven by hydrostatic pressure inside the cell, and the pressure is generated by contraction of actomyosin fibers. And inside the plasmodium tube, there thought to be highly oriented bundles of actomyosin fiber. We thus assume that the acceleration of locomotive velocity in experiment II can be explained by supposing that the tubes generate larger driving force, compared with the cellular mass or sheet-like locomotive front. A similar explanation is possible for the increase in morphological complexity in experiment I: along with the transplantation of tube and increase in the intracellular pressure, formation of filopodia-like structure may become dominant for emergent release of the pressure.

The plasmodium motility is known to be coupled with the patterns of cytoplasmic contraction (Nakagaki et al., 2000b). We also assume that the cytoplasmic contraction/oscillation is affected by arrangement of tube, and this would direct the cell motility, leading to the biases in experiment III. Such a hypothesis is yet to be studied, however, what important here is the fact that the tubes have an effect on the motility. As mentioned a few times, tubes are formed reflecting the history of cell motility. In this sense, the arrangement of the tubes is a record for the plasmodium motility. And if the tubes direct the future motility, at least partially, then the tubes are working as memory reservoir. In fact, the dependency of the motility on locomotive history seems to physiologically contribute to exploratory behavior of the plasmodium. In Figure 9, the plasmodium showed the directional bias in their motility, avoiding the direction that had already been explored. Of course, such behavior of the plasmodium is a kind of anticipation; the plasmodium anticipates an appropriate course for its movement referring to its past exploration. This is an internalist anticipation, because the plasmodium decides what to do in its future according to its history, but does not predict what comes next from the environment (Dubois, 1998).

In conclusion, we found some phenomena indicated that the plasmodium has memory reservoir. Symmetrically, the possible presence of the reservoir implied the presence of anticipatory aspect in the adaptive and explanatory cell motility. Future study will elucidate the mechanisms of the phenomena, and further investigation will be done in terms of computing anticipatory systems.

5 Conclusion

In our study on the relationship between the tubes and sheets of the plasmodium, we found that the arrangement of tubes plays important roles in determining the cell motility of the plasmodium. In fact, the presence of tubes promoted the heterogeneous morphology, accelerated the velocity of cell motility, and biased the direction of cell motility. The tubes are formed reflecting the history of locomotion of the cell, and in this sense the arrangement of the tubes is a record for the past activities of the plasmodium. Therefore the plasmodium possibly uses the tubes as a kind of memory reservoir, and in the future study we will test this hypothesis by investigating how the tubes direct the cell motility of the plasmodium.

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References

Adamatzky A., De Lacy Costello B. and Shirakawa T. (2008) Universal Computation with Limited Resources: Belousov-Zhabotinsky and Physarum Computers, Int. J. Bifurcat. Chaos 18, pp. 2373-2389.

Aono M., Hara M. (2008) Spontaneous Deadlock Breaking on Amoeba-based Neurocomputer, Biosystems 91, pp. 83-93.

Camp W. G. (1936) A Method of Cultivating Myxomycete Plasmodia, Bull. Torrey. Bot. Club 63, pp. 205-210.

Dubois D. M. (1998) Introduction to Computing Anticipatory Systems, International Journal of Computing Anticipatory Systems 2, pp. 3-14.

Nakagaki T., Yamada H, Tóth Á. (2000a) Maze-solving by an Amoeboid Organism, Nature 407, p. 470.

Nakagaki T., Yamada H. and Ueda T. (2000b) Interaction Between Cell Shape and Contraction Pattern in the Physarum Plasmodium, Biophys. Chem. 84, 195-204.

Nakagaki T., Yamada H, Tóth Á. (2001) Path Finding by Tube Morphogenesis in an Amoeboid Organism, Biophys. Chem. 92, pp. 47-52.

Nakagaki T. (2001) Smart Behavior of True Slime Mold in Labyrinth, Res. Microbiol. 152, pp. 767-770.

Nakagaki T., Yamada H. and Hara M. (2003) Smart Network Solutions in an Amoeboid Organism, Biophys. Chem 107, pp. 1-5.

Nakagaki T., Kobayashi R., Nishiura Y. and Ueda T. (2004) Obtaining Multiple Food Sources: Behavioural Intelligence in the *Physarum* Plasmodium, Proc. R. Soc. Lond. B 271, pp. 2305-2310.

Rasband W. S. (1997-2008) ImageJ, U. S. National Institute of Health, Bethesda, Maryland, USA, http://rsb.info.nih.gov/ij/

Shirakawa T. and Gunji Y.-P. (2007) Emergence of Morphological Order in the Network Formation of *Physarum polycephalum*, Biophys. Chem. 128, pp. 253-260.

Shirakawa T. Adamatzky A., Gunji Y.-P. and Miyake Y. (2009) On Simultaneous Construction of Voronoi Diagram and Delaunay Triangulation by Physarum polycephalum, Int. J. Bifurcat. Chaos 18, pp. 2373-2389.

Tsuda S., Aono M. and Gunji Y.-P. (2004) Robust and Emergent Physarum Logical-computing, Biosystems 73, pp. 45-55.

Tsuda S., Zauner K. P. and Gunji Y.-P. (2007) Robot Control with Biological Cells, Biosystems 87, pp. 215-223.