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# Basic Transitions of *Physarum Polycephalum*

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***Abstract*—The main charter of this work is the organism *Physarum polycephalum*, in particular plasmodium, *Physarum*'s vegetative phase. During this latter form, the organism is more active and moves searching for food. Plasmodium behaves like a giant amoeba, and more interestingly, its way of foraging can be interpreted as a computation. By comparing the reaction of this organism with attractors and repellents, knowing its capability of solving computational problems with natural parallelism, we dedicated the present work to study the behavior of *Physarum polycephalum* slime mold under different conditions.**

## I. Introduction

*PHYSARUM polycephalum's* behavior [1] has been the object of different studies belonging to different research fields, from biology to unconventional computing and from experimental to theoretical science. Thus, considering the sentence just mentioned, the question coming out is "Why does *Physarum* attract so many different scientists?"
It belongs to one of the predominant groups in the world of eukaryotes: Amoebozoa [2] a family hundreds millions of years old. Although they are probably one of the first forms of life on Earth, few are data about them because of the evolutionary variation happened across the years to this group. In this field, most of the interest of scientists is focused on slime molds [3]. *Physarum polycephalum*, in particular, can be considered as a reaction-diffusion medium enwrapped in a growing membrane. Moreover, the plasmodium of *Physarum* is simple enough to be modeled and implemented in unconventional computing algorithms as a non-linear media. However, on the other hand, it is a robust organism, reach of challenging features allowing many different computational studies [4].

In [5] *Physarum* has been compared to a labelled transition system whose behavior has been modeled by a rough set models, implemented in a specific language and properly developed to describe *Physarum polycephalum's* response to spatial configuration of stimuli [6]. Additionally, its foraging behavior has been related to computation [4], in fact, attracting/repelling sources are able to induce in it excitation waves, affecting its motion. Thus, because of the parallel processing of inputs and outputs, it can be seen also as a massive-parallel amorphous computer, taking data from food sources in the space around and giving, as a result, the protoplasmic network created by its own body [7]. A recent example of *Physarum's* capability of designing optimized networks, allows the retracing of Canadian Highway Networks [8]. Additionally it has been developed an algorithm [9] able to find the shortest path, as slime mold naturally does. Among all these feature, its behavior can be resumed as: capability of optimizing shortest path [10], calculation of proximity and planar graphs [11], calculation of basic logical operation [12], motion control [13], self-adaptation [14], and self-reparation [15].
This paper shows experiments done to study the behavior of this organism under different conditions and stimuli. In particular, we analyzed not only spatial distribution of attractants/repellents, but also the effect of external elements inserted and transported by *Physarum polycephalum*. Therefore, considering the obtained results, we can say that it should be possible to program this transition system [5] to realize deterministic adaptive networks and spatial distribution of nanoscale and microscale materials. Choosing, among transported materials, those able to change electrical, optical and, magnetic properties of the system it should be possible to enable information sensing and processing. The work is organized as follows: the first section analyzes *Physarum*'s behavior in presence of rose and geranium, while the second one is dedicated to chemical compounds. Then, we distributed food around the experimental area in a particular way, like the hours of a clock, in order to see how would appear *Physarum*'s realized network. The fourth study is devoted to light, a strong repellent for the mold, able also to induce sclerotization, its dormient phase, assumed in case of not proper environment conditions [16]. The fifth section presents the reaction of *Physarum* to music and sounds of different frequencies. As was already presented in [17] there is a correlation between

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motion of plasmodium and the music or sound frequency. Last section describes the experiments performed using *Physarum* as a bio-robot, able to transport microparticles whithin its own body, as in [18]. In particular, we worked with polystyrene and $MnCO_3$ microparticles.

The results here reported can be useful for unconventional computing and bio-computing application devices. In such field, *Physarum polycephalum's* networks become the scaffolds of hybrid circuits at the micro and nano-scale. For the development of self-growing computational systems, we must consider and study the slime mold as a medium capable of integrating and redistributing foreign particles. Therefore, *Physarum polycephalum* acts as a programmable transport medium as showed in [19].

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## II. EXPERIMENTS

*Physarum polycephalum*'s colony has been grown starting from sclerotized samples. The organism was fed with oat-flakes and kept in a dark and humid chamber in 9 cm diameter Petri dishes with 1,5% Agar non nutrient gel. Moreover, in order to allow a proper grown and safety conditions for the colony itself, the organism was periodically replanted to fresh Agar Petri.

### A. Biological elements

In wild nature *Physarum polycephalum* can be found in the underwood, near leaves, where the sun light is partially shielded by the trees. Therefore, it is reasonable to think that the mold could meet, during its motion, elements like flowers, leaves, grass, etc.

Fig. 1 Optical microscope photograph of a Petri dish with *Physarum polycephalum* growing networks firstly in the direction of the geranium.

In this section, we used as biological elements, geranium's leaves and rose-hip petals. Photographs in Fig.1 and Fig.2 show two similar experimental set-up. *Physarum polycephalum* was in the middle of a Petri dish, with Agar non nutrient gel, and starting from an oat-flake. Then, we distributed oat flakes along the perimeter, but near two of them, we added respectively: geranium's leaves in the first case and rose-hips petals in the second. The experiments have been developed keeping the set-up in the dark and humid chamber exploited for maintaining the colony and taken out of just for checking. After 10 hours, *Physarum* reached the oat-flakes near the leaves or petals, as shown respectively in Fig. 1 and Fig. 2. Repeating each case 10 times, we verified that mold's behavior was always the same. Therefore, we can conclude that both geranium and rose-hips work as attractors for *Physarum*.

Fig. 2 Optical microscope photograph of a Petri dish with *Physarum polycephalum* growing networks firstly in the direction of rose-hip petals.

### B. Chemicals

In this experimental section, we studied slime mold's behavior in presence of two polysaccharides compounds, able to create a gel: pectin and chitosan. Firstly, we prepared the samples by dipping a circular glass, 10 mm in diameter, in the polysaccharide solution. The latter when drying forms a homogeneous gel layer on which we posed a small blob of *Physarum.* Then, the so created sample was positioned into a Petri dish with Agar non-nutrient gel, oat-flakes were spread around the glass to push *Physarum* growing its networks on the substrate and reaching them. As in the case of biological elements, the experimental Petri dishes were kept in the dark and humidity. We developed the same set-up for chitosan and for pectin; in both cases and for all the experiments, we got positive results, i.e. the mold survived creating networks towards the food. Fig. 3 shows one of the chitosan experiments performed, after one night. The mold reached the food surviving and forming protoplasmic tubes across the substrate, thus, proving the biocompatibility of chitosan for *Physarum*.

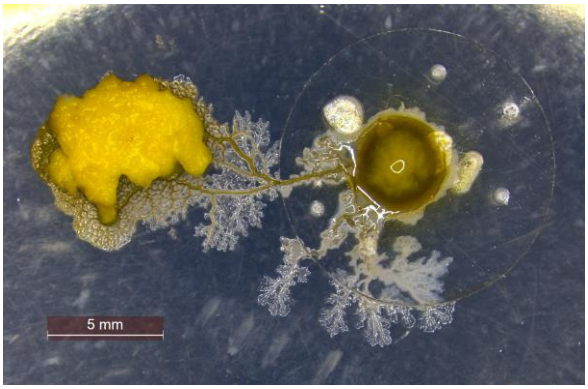


Fig. 3 Optical microscope photograph of a glass with chitosan from which the mould started its motion

Fig. 4 shows an experiment performed with pectin. The sample has been photographed after 14 hours; *Physarum* created networks and veins of protoplasmic tubes going out of the glass and reaching the oat-flakes. Therefore, also pectin can be considered a biocompatible element for slime mold.


Fig. 4 Optical microscope photograph of a glass with pectin from which the mold started its motion

### C. Clock

We developed this series of experiment in order to understand if *Physarum* has a preferential direction, and how its networks will appear, considering a so particular food distribution.

These are three sequential optical microscope photographs, see Fig. 5, of the same sample defined as *clock*. Pictures refer to three times interval, after 24, 48 and 72 hours respectively.

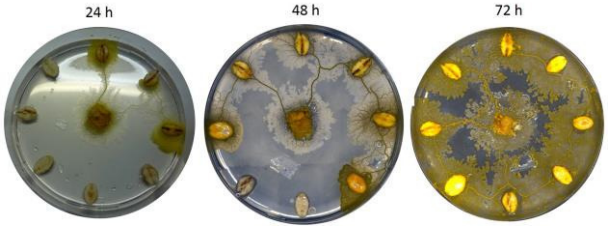


Fig. 5 Photograph of the same sample after 24h-48 and 72 hours of *Physarum* growing on agar non nutrient gel with oat-flakes distributed like hours in a clock

The mold started from the middle of the Petri, placed on an oat-flake; then, after disposing all other oats along the perimeter, like clock's hours. The sample was kept in a dark and humid environment for the whole time, and just photographed at the time-break abovementioned. As it is visible from the first Petri (i.e. after 24 hours), *Physarum polycephalum* created 2 protoplasmic tubes in the direction of two neighbors oats and, than it grew at the same time in the two opposite directions. Fig. 6 is another photograph of *clock* experiment after 12 and 24 h.

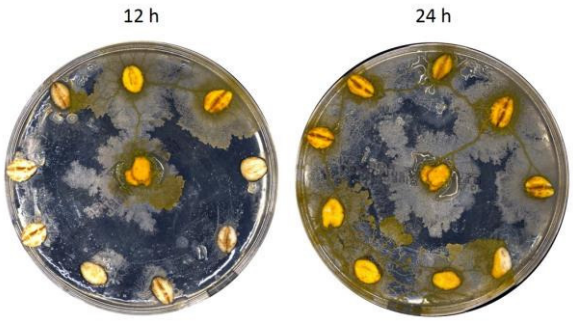


Fig. 6 Photograph of a sample in the clock series after 12 and 24 hours

Fig. 7 is another experiment of *clock*, but it is different from previous cases. Here oat-flakes have been placed closer to the starting point (in the middle of the Petri) and, as it is clearly visible, *Physarum* started on three oat flakes.

Fig. 7 Photograph of a sample in the *clock* series, in this case mold started from three oat-flakes in the middle of the Petri. Other flakes have been placed closer than in other cases.

What we got after all these experiments was that Physarum seems to have a preferential direction, on right, as many animals and insects [20]. However, there is still not statistical significance about these right decisions.

### *D. Light*

In Fig. 8 we worked with light and *Physarum*, studying the repellent effect that the first has to the latter. Starting from the generally used Petri with Agar gel inside, we masked with a black tape the outer part of the Petri dish, leaving transparent just an area with an *S* shape. Therefore, we placed a cold light source underneath the Petri and developed the experiment. What stands out from Fig. 8 is that *Physarum*, from its starting point moved in the food direction avoiding the illuminated area of the Petri.


Fig. 8 Photograph of a sample in which has been tested the repellent effect of light on *Physarum polycephalum*

### *E. Music*

The Photograph of Fig. 9 presents a series of three Petri Dishes with the same set-up: agar non-nutrient gel, the same quantity of *Physarum* starting from the middle and 4 oats placed at 45 degree one with respect to the other.

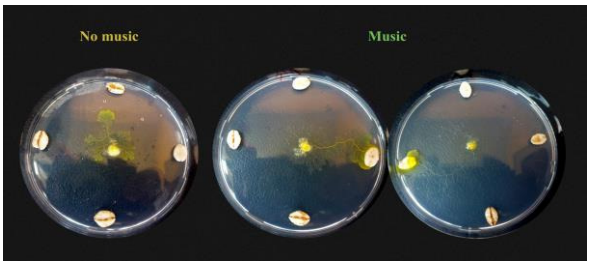


Fig. 9 Photograph of three sample in which the effect of Mozart's music on the mold's speed has been tested

Therefore, two of these samples were placed near a sound source, in particular Mozart symphony; the third one was in a silent place. What we observed, not only in these cases, but in all the so developed experiments, was that the frequencies of Mozart's music have a positive effect on *Physarum polycephalum* increasing its speed. Moreover, we performed other experiments changing kind of music, in particular hard rock songs (ACDC and Iron Maiden) and heavy metal (Carribean Corps and Marilyn Manson). It was interesting to verify that, in case of hard rock, there was not appreciable difference in the speed of *Physarum* samples under music with respect to those in a silence environment. However, when the mold was exposed to heavy metal, the sound resulted in the fact that the organism reduced substantially its speed, and in a 10% of cases, the whole motion was interrupted and it starts sclerotizing. For this study, we repeated each experiment ten times placing in parallel a sample under music and another under silence, thus keeping the same surrounding conditions.

### *F. Microparticles*

We loaded *Physarum polycephalum* slime mold with two kind of microparticles:

1_ Polystyrene microparticles (bought by Sigma-Aldrich), an aqueous dispersion (% 0.5 p/p) of particles 3 μm in diameter.

2_ $MnCO_3$ microparticles (bought by Plasma Chem., Berlin) with a diameter of 3 μm in aqueous dispersion (% 0.5 p/p).

3_ $BaFe_{12}O_{19}$ nanoparticles (obtained by a sol-gel method [14]) were solved in a homogeneous suspension in pure water with a final concentration equal to 2 mg/L. The solution was sonicated before its deal.

The performed experiments had, as an objective to verify the compatibility and the ability of the mold to transport

micro and nan-objects, of different nature. Microparticles were chosen because of their nature, in particular organics, polystyrene, and non organics, $MnCO_3$. The latter, nanoparticles of Barium hexaferrite were selected because they are magnetic, a necessary feature to develope the experiments we were planning to carry out.

Additionally, we studied two kinds of transport: first case we loaded *Physarum* with particles, by directly mixing the two elements together; in the second one, we observed what happened if *Physarum* had to cross a strip of particles. The latter study, however, was performed only with polystyrene microparticles. Moreover, it has to be distinguished two main transport mechanisms: in a first case, particles are transported on *Physarum* veins, in a second one, they are taken inside the body of the mold. The first case is typical of those substances that *Physarum* almost ignore; they are not dangerous, nor interesting for it. Thus, they are transported just because, during its motion, they sticked to its body, but they are not incorporated. Therefore, it is possible to find them only on the surface of mold's body, and not inside it. In the second case, particles are mistaken as food, so they are engulfed inside *Physarum* that picks them up and tries to absorb.

We proceeded in parallel with both micro-particles (polystyrene and $MnCO_3$) solutions: 20–30 μL of particles were directly added to the plasmodium and mechanically mixed. The so obtained solution, of *Physarum*-particles, was placed on a silicon substrate. To induce the organism in moving and, to understand if it is able to transport these object, and in which way, we placed oat-flakes on a second silicon substrate, posed next to the one the mold was starting from. Then, we placed samples in a dark and humid environment and, after 12 hours, loaded *Physarum* not only survived, but, creating networks of protoplasmic tubes, reached also the food on the second substrate. The latter was used to perform scanning electron microscopy (SEM) analysis, after sclerotizing the network by light illumination. In this way, we are sure that the particles, we will found, have to be transported by *Physarum.*

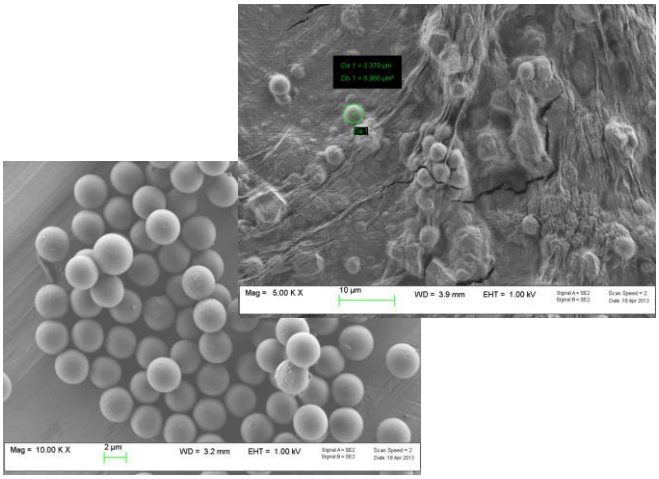


Fig. 10 Two SEM pictures: the one on left depicts polystyrene microparticles, while, on right, there is a sclerotic *Physarum* vein loaded with polystyrene microparticles by mechanically mixing method.

The right picture of Fig. 10 shows polystyrene particles within *Physarum*'s body. We found particles inside and on the surface of the mold, thus, *Physarum* incorporates and integrates polystyrene micro-objects. Moreover, we proved also that *Physarum* transports these materials, bringing them actively from the starting point, where they have been mixed, to the point the mold moved to in order to reach the food.

Figure 11 shows SEM measurements of a sclerotized vein of *Physarum* and $MnCO_3$ microparticles. The latter are clearly visible within the crack and near the veins of the mold.

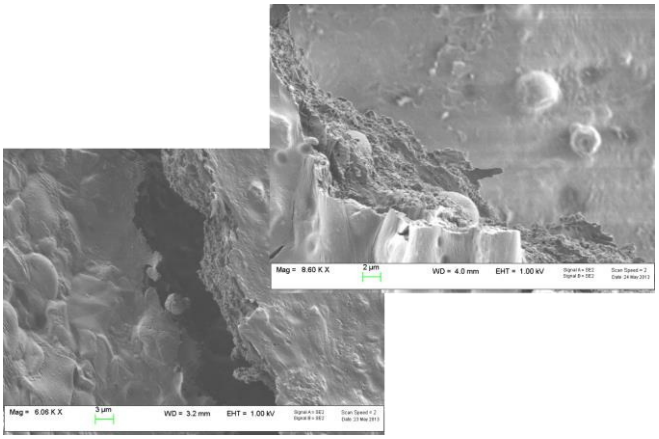


Fig. 11 SEM measurements performed on the sclerotized *Physarum* veins charged with MnCO3 particles. Also in this case the analyzed samples are those where *Physarum* moved to after loading. In both pictures particles are distinguishable within veins and cracks of the mold, in addition there are also two particles near the cut vein (right picture).

We performed a second type of study, just considering the polystyrene microparticles. Creating a strip of particles and an "ad hoc" experimental set-up, we forced *Physarum* in crossing particles' strip. Moreover, starting from a rounded glass, placed into a Petri dish with Agar non-nutrient gel, we designed a strip, one mm width, with the help of kapton, in a way that finally the strip divided the glass in two half. At this point, we placed a 2 µl of *Physarum* blob on one-half of the glass. However, in order to force *Physarum* in crossing the strip and not reaching the food, beyond the particles, by alternative paths, we created a semicircle with repellent agar, all around the mold. Therefore, the only possible way for *Physarum* to reach the food was through the particles strip. Fig. 12 shows that *Physarum polycephalum,* from the starting point, created protoplasmic tubes in the direction of food and, during its motion, crossed the strip made by particles. As in the previous cases, the mold transported particles that were found inside its slime after its crossing.

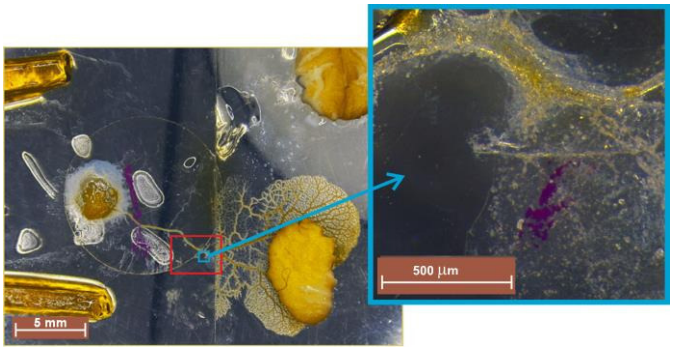


Fig. 12 Optical microscopy photograph of one of the developed experiment with slime mold and red polystyrene microparticles.

Last series of experiments have been performed with magnetic nanoparticles. In order to be able to recognize them once inside the mold we analyzed firstly the nanoparticles solution deposited on silicon substrate as in Fig.13

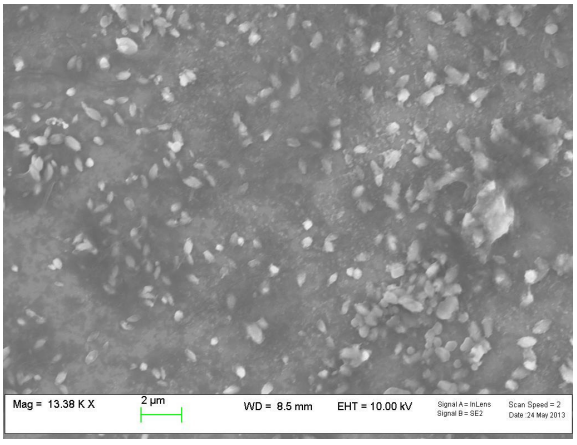


Fig. 13 SEM image of $BaFe_{12}O_{19}$ nanoparticles on silicon substrate

Slime mold was loaded with barium hexaferrite nanoparticles by mixing 100-200 µL of *Physarum* with 20-30 µL of the magnetic particles suspension.

Magnetic nanoparticles typically form big aggregates, due to the magnetic interactions, being ferromagnetic at that size. As in case of microparticles, we studied the efficiency of the slime mold loaded with magnetic nanoparticles. Firstly, we verified that the mixing procedure of the slime mold, with these particles, did not result in the organism death. As we saw, the slime mold kept its activity for more than a month, we focused the experiment on *Physarum*'s capability of transporting nanoparticles during its growth, proceeding exactly as in the case of polystyrene and $MnCO_3$ microparticles. However, even if *Physarum* moved to the second substrate, as we expected, a direct SEM imaging (Fig. 14) did not allow visualizing the presence of particles, due to their dimension that can be mistaken with nuclei of *Physarum*. For this reason, we fixed particles on the substrate surface and removed the slime mold

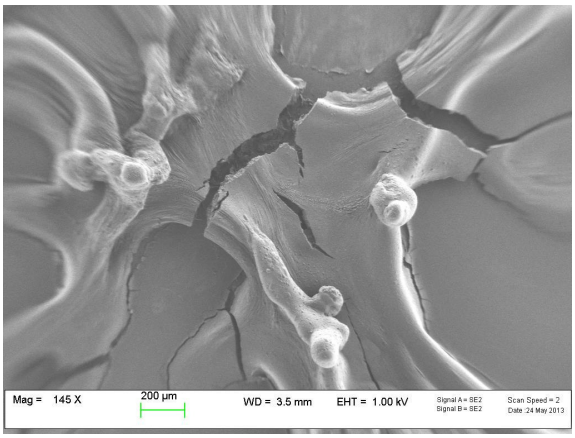


Fig. 14 SEM measurement of a sclerotized vein of Physarum polycephalum loaded with magnetic nanoparticles $BaFe_{12}O_{19}$. What stands out from this picture, as in the other done with such kind of particles inside the mold, are particular and curious 3D structures. The latter have been observed only in the samples when *Physarum* was loaded with magnetic nanoparticles of $BaFe_{12}O_{19}$

Considering that particles are magnetic, we decided to fix them by means of an external magnet placed under the sample where *Physarum* moved to by networks formation. Therefore, particles would be attracted by the magnet downwards and kept attached to the sample surface during the removal of the slime mold, by washing. Finally, the sample, always keeping the magnet attached, was dried and analyzed by SEM measurements. In order to show that the elements are really connected to the particles, element mapping was performed. The appropriate SEM image is shown in Fig. 15.

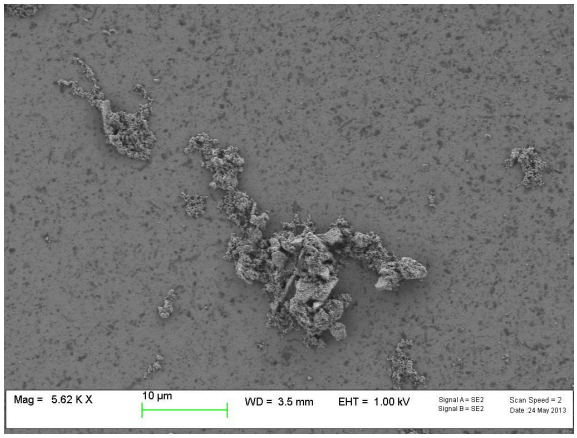


Fig. 15 SEM image of tubes formed by *Physarum* during its growth towards the food and subsequently washed, as described in the text.

## III. CONCLUSIONS

In this work we studied the behavior of *Physarum polycephalum* as an organism able to realize natural transition systems [4], *Physarum* is an interesting starting point for sensing, computing, novel biological substrate formation and circuit design. The possibility of targeting the growth with external disposition of attractants and repellents provides new capabilities for the realization of bio-inspired computational or robotic systems, based on *Physarum polycephalum*. In fact, such action can accelerate and/or suppress the propagation in certain directions. In order to illustrate this statement, let us consider a slime mold growth on the support, supplied with position sensors (in the simplest case – just electrodes, varying the capacity when the slime mold arrives to them). Moreover, analyzing *Physarum's* position, it will be possible to dispose attractants and/or repellent in such a way that the sequence of the food sources, where the slime mold will arrive, can be predetermined. By complementing with colored food [19], it will be possible to make any desirable mixture of colors. Of course, instead of the color, the food can include substances that, transported in special zones, evolve in some desired reactions. The final product will depend on the sequence of the reagents mixing. Thus, the *Physarum* will act as a bio-robotic system to delivery and control chemical reactions. The attractive and repellent parameters, such as the presence of food, light, humidity, chemistry of the surfaces, can be considered as inputs of this biocomputing/actuating system. The final distribution of the grown tubular pattern shows the system's output. The increase of the number of the control stimuli can improve the computational capabilities [11] of slime mold, especially if there will be a possibility of targeting the growing active zones of slime mold with robust external stimuli. The possibility, of targeting the growth with external actions, provided by author [21] serving *Physarum* also as a bio-living-robot, opens new capabilities for the realization of bio-inspired computational or robotic systems based on the slime mold. Future works involve the feeding of *Physarum* slime mold with nanoparticles that would be adsorbed in different way, depending on the biocompatibility; another field, that could be considered related to the previous one, concern nanoengineered polymeric capsules with shell made by mold-attractants, but filled with some solutions not so appealing for the bio-robot. These ideas open a huge amount of experiments, studies and analysis.

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