Flow-network adaptation and behavior in slime molds

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ABSTRACT

The slime mold *Physarum polycephalum* is an amoebozoa that grows forming a cytoplasm network that adapts its geometry to external stimuli. The cytoplasm is made of ectoplasm tubes in which the endoplasmic fluid flows. Endoplasmic flow is due to the rhythmic contraction of the actomyosin fibers of the ectoplasm, which induces a peristaltic wave that can be tracked through the spatiotemporal variations of the tube diameters. Slime mold behavior depends on many periodic modes of tube diameter variation, which is believed to allow a smooth transition between migration directions. *Physarum polycephalum* can solve mazes and grow optimal networks to solve traveling salesman and Steiner tree problems. Slime mold network dynamics have been modeled through cell automata and stochastic approaches, as well as fluid flow equations, electronic analogs, and multi-agent systems. Here, we examine the modeling strategies available to date to simulate flow-network adaptation in slime molds. However, we found no theoretical framework that can properly predict the evolution of the network as it morphs from an initial configuration to a pseudo-asymptotic optimum or explain the physical phenomena that drive endoplasmic flow or memory encoding at the scale of the entire network. Multi-frame object tracking by k-partite graphs holds promise for slime mold network analysis and tracking, whereas deep learning could be used to classify sequences of latent features to help characterize the behavior of *Physarum polycephalum*. The combination of the two could pave the way to a new class of predictive behavior models for slime molds.

1. Introduction

Since the seminal contribution of Toshiyuki Nakagaki twenty years ago (Nakagaki et al., 2000), the acellular slime mold *Physarum polycephalum* has become an essential model organism for studying problem-solving in non-neural systems. For example, past experiments have shown that this organism can find the shortest path in a maze (Nakagaki et al., 2000), build optimized networks to connect several food sources (Evangelidis et al., 2017; Kay et al., 2022; Tero et al., 2010), anticipate events (Saigusa et al., 2008), learn to ignore irrelevant stimuli (Boisseau et al., 2016; Boussard et al., 2019; Dussutour, 2021), encode memory in their environment (Reid et al., 2012) or in their morphology (Kramar and Alim, 2021), interact and exchange information with their congeners (Vogel et al., 2015; Vogel and Dussutour, 2016; Briard et al., 2020; Rolland et al., 2023), optimize nutrient intake (Dussutour et al., 2010), and make optimal (Beekman and Latty, 2015; Reid et al., 2016) or irrational decisions (Latty and Beekman, 2010). P. *polycephalum*'s behavior relies on its self-organized internal architecture, which consists of a transport network of interconnected tubes

(Fleig et al., 2022). In this review paper, we summarize the current state of the research on the environmental stimuli that affect the geometry of that network, the types of behaviors observed in correlation to the network geometry, and the persistence of the network geometry after the behavioral response to a stimulus. In the following, behavior refers to variations of fluid flow in the network responsible for movement induced by metabolic functions (e.g., nutrient search or absorption), defense mechanisms (e.g., avoidance of adverse environments or organisms) or synergetic principles (e.g., fusion between two or more cells). One of the key questions that motivates this study is whether or not the network encodes past experiences, which could be seen as a form of cell memory.

Although significant research has been done in recent decades, our understanding of acellular slime molds and their role and interactions within ecosystems is still incomplete. P. *polycephalum* is distributed worldwide and can be found in a variety of microhabitats, including coarse woody debris, ground litter, aerial litter, and the bark of living trees (Novozhilov et al., 2022). It thrives in moist, moderate temperatures, and requires sufficient decaying organic matter to grow

(Novozhilov et al., 2022). It feeds on protists, fungi, and bacteria. P. *polycephalum* has frequently been used as a model system due to its ease of cultivation in the laboratory, and its behavior can be extrapolated to other myxomycetes (Oettmeier et al., 2022). The life cycle of slime molds has been discussed in length in (Keller et al., 2022). Here is a quick overview. The vegetative state of *P. polycephalum*, the plasmodium, is a giant mobile cell, whose basic structure consists of a syncytium of nuclei and a complex cytoplasmic network of veins (Fig. 1). Microplasmodia, the smallest viable units, range from 50 to 500 μm in size while macroplasmodia can extend up to hundreds of square centimeters (Oettmeier et al., 2018). A plasmodium can be severed into viable and structurally similar yet smaller subunits. Upon contact, plasmodia can fuse with each other, giving rise to the (re-)formation of a plasmodium. A starving plasmodium can encapsulate and enter a dormant stage called sclerotium and can turn back from this resting stage to a plasmodium within 24 h after transfer to fresh food medium. When the plasmodium reaches maturity, it forms fruiting bodies which release spores that are dispersed by air and animals. Under favorable conditions, these spores germinate and give rise to either a myxamoeba or a swarm cell depending on environmental conditions. Under adverse conditions, these cells may form a microcyst, whereas under favorable conditions, they may fuse with genetically compatible types and develop into a new plasmodium.

In the following, we first review the causal links between environmental stimuli, slime mold network and slime mold behavior. Secondly, we highlight P. *polycephalum* network optimization properties to cope with changing environmental conditions. Next, we examine the modeling strategies available to date to simulate slime mold networks in a variety of environmental conditions. We then propose research perspectives to better understand the timing of P. *polycephalum* network geometry adaptation to external stimuli and subsequent behavior. An important question that remains unanswered is whether or not network optimization leads to behavior optimization in terms of species survival at the scale of a single cell or at the scale of a group of cells. We conclude by summarizing the most intriguing findings and most pressing questions to date.

2. Slime mold network: biological description

Slime molds are intricate systems composed of interconnected and interacting networks, such as intracellular signaling, cytoskeleton, receptor, and genetic networks (Oettmeier et al., 2022). Here, we will focus on the tube network as it embodies perfectly the concept of a network in slime molds, an undirected graph where edges are tubes and nodes are branching points. In *P. polycephalum*, motion relies on a transport network consisting of interconnected tubes (also called veins in the following) that form the cytoplasm. The ectoplasm, the visco-elastic part of the cytoplasm, forms the contractile walls of the tubes (Fig. 1). Within these tubes flows the endoplasm, the liquid part of the cytoplasm, which contains all organelles including nuclei and mitochondria. The ectoplasm and endoplasm have similar composition and can be converted into one another (Isenberg and Wohlfarth-Bottermann, 1976). This compartmentation between ectoplasm and endoplasm is due to the organization of actin and myosin, the fibrous and contractile proteins that form the cytoskeleton of the slime mold. The endoplasm is converted into ectoplasm upon polymerization of actin. The endoplasm travels through the ectoplasm via internal veins that appear and disappear based on local pressure and shear (Guy et al., 2011). Unless the endoplasm is moving, it is difficult to distinguish between the two fractions. The veins allow respiratory gases, molecules and organelles to be exchanged with the surrounding cytoplasm. This open network allows homeostasis to be maintained in plasmodia ranging from 10 μm^2 to 10 m^2.

The spatial structure and the dynamic behavior of the network allow an efficient regulation of the cytoplasmic flow within the entire plasmodium and play a fundamental role in homeostasis (Bottermann et al., 1983; Korohoda et al., 1983). The endoplasm flows passively within the tubes, while the ectoplasm undergoes a cycle of active contractions and relaxations (Wohlfarth-Bottermann, 1979; Boussard et al., 2021). This active process is driven by the combination of force generated from actomyosin activity (Naib-Majani et al., 1984; Ishigami, 1986; Stockem and Brix, 1994) and chemical regulation, involving several substances including Ca^{2+}, NADPH, H^+ and ATP (Ridgway and Durham, 1976; Kuroda and Kuroda, 1982; Ueda et al., 1987; Nakamura and Kohama,
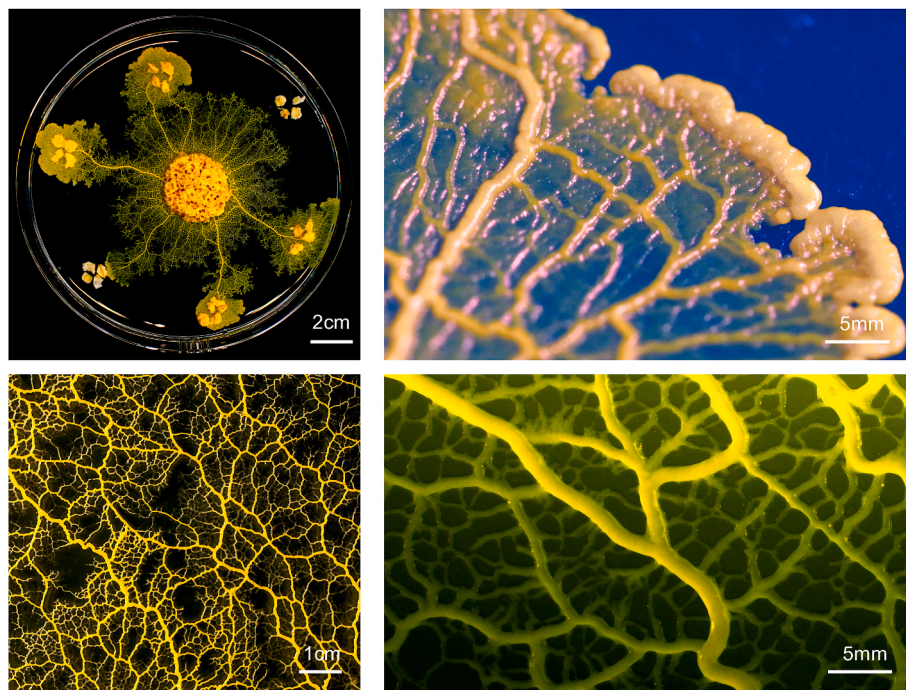

Fig. 1. Pictures of *Physarum polycephalum* networks. Top left: A macroplasmodium extending its network to contact various food sources. Top right: A fan shaped exploring front followed by a network of vein. Bottom left and right: close picture of the vein network. © Audrey Dussutour.

1999; Yoshiyama et al., 2010; Teplov, 2017; Boussard et al., 2020). Local cross-sectional contractions along a vein induce a pressure difference, which in turn leads to endoplasmic flow (Alim et al., 2013; Lewis et al., 2015; Rieu et al., 2015). Contractile actin fibrils found in the ectoplasm are probably the key players in these cross-sectional contractions (Lewis et al., 2015; Rieu et al., 2015) which travel from the rear to the front.

A unique aspect of *P. polycephalum* endoplasmic flow is the rhythmic reversal of flow direction. This process is known as shuttle streaming (Tyson, 1982), and it is generated by a peristaltic wave with a wavelength corresponding to the size of the network (Alim et al., 2013). The contraction patterns repeat every 90 s (Coggin and Pazun, 1996), and the flow within the veins reverses on a similar timescale. *P. polycephalum* displays oscillations of approximately the same period but different phases in various parts of the plasmodium (Julien and Alim, 2018). Interestingly, the shuttle streaming itself affects the contraction rhythm as it is believed to be necessary to synchronize the contractions (Yoshimoto and Kamiya, 1978), and insufficient streaming alters the synchrony between the contractions and the cytoplasmic flow (Nakagaki and Ueda, 1996). On top of shuttle streaming and peristaltic flow, other movements observed in slime molds include standing waves, synchronous pulsations, quasistochastic oscillations, and wave-like thickness oscillations (Kobayashi et al., 2006; Takagi and Ueda, 2008; Rodiek et al., 2015; Teplov, 2017).

3. Slime mold network: automated extraction

Slime mold shape and network that has been discussed so far have been extracted using various automated methods (see e.g Vogel et al., 2015; Fricker et al., 2017; Patino-Ramirez et al., 2019, 2021). Fig. 2 shows an example of slime mold network analysis that relies on the conversion from pixel to graph. Usually, image analysis tools are developed for a particular experiment, but can be used for different settings or model systems (Baptista and De Bacco, 2021). Most programs can be used to extract shape descriptors such as, among others: cell area, perimeter, aspect ratio, circularity, fractal dimension of the perimeter, polarization and number of exploring fronts. Network quantifiers that have been extracted successfully include metrics such as the probability density functions of vein radius and length distributions, angles at bifurcation, redundancy, and node connectivity. At the intracellular level, the actomyosin cortex underlying the vein network has been imaged using phalloidin, a peptide used for staining actin filaments, but only for static analyses, i.e., at fixed times (Oettmeier et al., 2018). In the future, it would be interesting to extract the actomyosin network in vivo to understand its dynamic.

4. Slime mold network change in response to external stimuli to allow adaptive behavior

The vein network is responsible for cellular motility. *P. polycephalum* can migrate at a speed of up to 4 cm h^{-1} (Kessler, 1982) through the interplay of intracellular flow, adhesion and rhythmic contractions of the intracellular actomyosin cytoskeleton (Boussard et al., 2020). These contractions produce a pressure gradient that pushes the endoplasm towards the cell periphery, where the veins vanish and the endoplasm can flow freely (Teplov et al., 1997). Local cytoskeletal reorganization and local alteration of the actin–myosin cortex lead to the formation of pseudopods or fan-shaped leading fronts which extend and retract in synchrony with the shuttle streaming of the endoplasm (Oettmeier and Döbereiner, 2019b). In a homogeneous non-nutritive environment, slime molds grow isotropically over short distances, and then switch to a directed digitated growth (Vogel et al., 2015). In the presence of environmental stimuli such as a light source or chemicals, slime molds show directed movement toward or away from the stimulus. Indeed, upon external stimulation, *P. polycephalum* responds first by adjusting its oscillation frequency and amplitude locally. Positive stimuli lead to increased frequencies, whereas negative stimuli lead to decreased frequencies (Matsumoto et al., 1986; Miyake et al., 1994; Ray et al., 2019; Boussard et al., 2021). This change spreads over the entire network (Alim et al., 2017) and as a result, *P. polycephalum* adapts its morphology to the environment (Latty and Beekman, 2009; Patino-Ramirez et al., 2019) and migrates towards or away from a variety of external stimuli such as chemicals (Durham and Ridgway, 1976), light (Marwan, 2001), temperature (Wolf et al., 1997), humidity (Rakoczy, 1973), gravity (Block et al., 1994) or substrate distortion (Murugan et al., 2021).

The transport network created by *P. polycephalum* is hierarchical in nature with a few long veins with large radii that branch off in many thin veins (Baumgarten and Hauser, 2013). Thin vein diameters are related to the diameters of the larger veins by Murray's Law (Akita et al., 2016), which was initially proposed to explain the diameters of branching vessels in hemodynamics. The larger veins are usually more directional and form the foundation of the network, while the thinner veins establish local connectivity. The diameter of a vein increases with the flux that occurs in that vein (Baumgarten and Hauser, 2013). Like any other
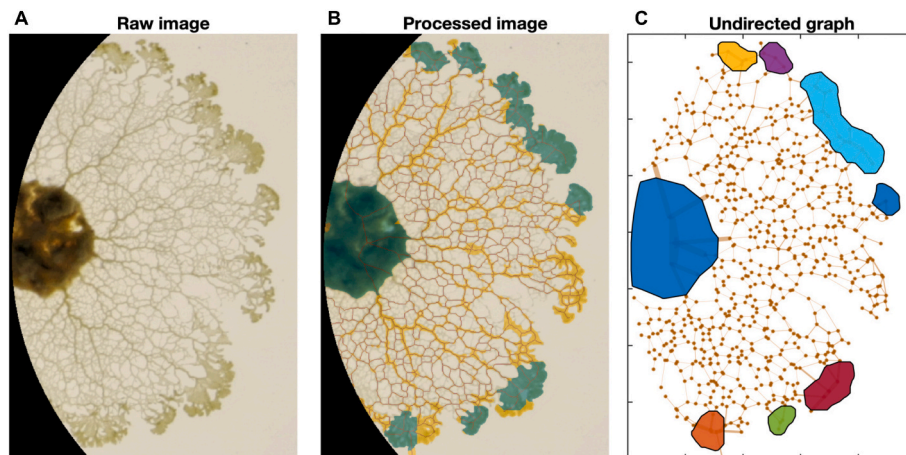

Fig. 2. Segmentation process. (A) Raw image, masked at the boundary of the petri dish and enhanced used a Laplacian filter. (B) Segmented image overlay on original image, with highlighted pseudopods and cell topological skeleton. (C) Resulting undirected graph: dots and segments correspond to nodes and edges respectively. Approximate location of clusters shown as colored polygons. Three videos exemplifying the time-lapse segmentation process are available as supplementary material of this study. Note: this figure was extracted from Patino-Ramirez, F., Arson, C. & Dussutour, A. Substrate and cell fusion influence on slime mold network dynamics. *Sci Rep* **11**, 1498 (2021). https://doi.org/10.1038/s41598-020-80320-2, which is licensed under a Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/).

network, the slime mold network consists of a set of nodes that are connected by a set of edge links. The nodes and edges form a non-directed graph. Like in most biological networks, the average degree of a node (i.e., the average number of nodes that any given node is connected to) in *P. polycephalum* networks is close to three, with only slight variations due to end branches (Dirnberger and Mehlhorn, 2017). Veins with larger diameters and lengths have higher centrality than those adjacent to them (Baumgarten et al., 2010; Patino-Ramirez et al., 2021), i.e., the shortest paths between nodes of the slime mold network have a higher probability to pass through the veins that are wider and/or longer. Smaller, less central veins bring adaptability and redundancy to the network, which is constantly changing (Dirnberger and Mehlhorn, 2017; Fricker et al., 2017). The continuous adaptation of veins in slime mold networks is thought to be driven by the shear stress applied by the endoplasm on the walls, which causes a reorganization of the cytoplasm protein fibers. According to the Hu and Cai model (2013) those fibers could change alignment relative to the axial vein direction, resulting in a variation of wall thickness. This adaptation (local dilation or shrinkage) could be related to the optimization of energy consumption of the network (Hu and Cai, 2013). Recent work by Alim's group (Marbach et al., 2023a, 2023b) highlights the effect of shear stress and subsequent shear rate on vein radius. The analysis is based on Newton's second law of motion applied to an infinitesimal vein segment, where the vein wall is subjected to hydrodynamic forces, potential forces due to the stored elastic energy in the tube, tensile forces induced by the actomyosin fibers, radial forces caused by the rearrangement of fibers under shear stress, and friction forces. The authors arrive at a first-order time differential equation for the vein radius (Marbach et al., 2023a):

$$\frac{d\langle a\rangle}{dt} = \frac{\langle a\rangle}{t_{adapt}}\left(\frac{\langle\tau\rangle^2}{\tau_0^2} - 1\right)$$

where a is the vein radius, $\langle...\rangle$ stands for a time average for a short timescale (e.g., quasi-instantaneous elastic deformation timescale), t_{adapt} is a timescale that characterizes adaptation, τ is the shear rate and τ_0 is a steady state shear rate. The equation above, the terms of which are illustrated in Fig. 3, can be solved over several time ranges to emphasize short-term fiber rearrangement or longer-term vascular adaptation. For instance, the effect of the shear stress induced by endoplasmic flow on the ectoplasm are seen after a time delay of 1–3 min. Marbach and collaborators (Marbach et al., 2023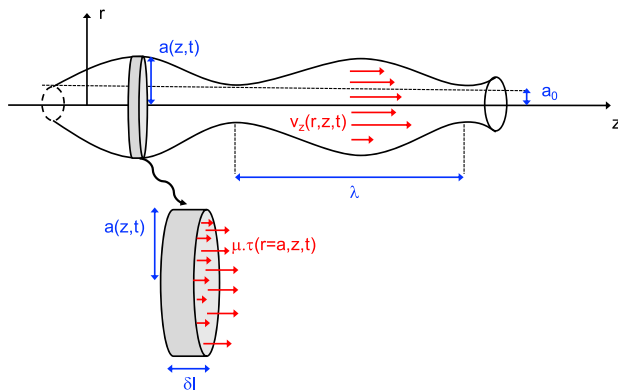a) also showed that vein fate depends on parameters that are based on the overall network architecture – such as local pressure or relative vein resistance. To date, this mechanistic model was not applied to the extension or retraction of the dangling end of a vein.

Nutrients and repellents affect the topology of the network formed by *P. polycephalum* (Takamatsu et al., 2009; Ito et al., 2011; Patino-Ramirez et al., 2021). For instance, localized chemical attractants can create changes in vein diameters which could be caused by a softening agent (Kramar and Alim, 2021). Recent results show that slime molds build sparse networks with thin veins in a neutral environment and more compact networks with thicker veins in a nutritive or adverse environment (Patino-Ramirez et al., 2021). They also revealed that slime molds construct long, efficient and resilient networks in neutral and adverse environments, whereas in nutritive environments, they build shorter and more centralized networks (Patino-Ramirez et al., 2021). In recent studies, it has been demonstrated that the slime mold *P. polycephalum* is able to store memories of food source locations through tube diameter hierarchical organization (Kramar and Alim, 2021) and could also store the responses to many stimuli (Bhattacharyya et al., 2022).

The dynamics of the network, the shuttle streaming and the resulting oscillation patterns are all connected to the behavior of *P. polycephalum*. However, to date, the relationship between the actomyosin system, the motive force generation, the network topology and the expression of a behavior in plasmodia of *P. polycephalum* remains elusive, in that no model can both predict and explain the adaptive behavior as a result of network evolution under external stimuli.

5. Slime molds optimize their networks

P. polycephalum can build a network that extends on several centimeters and can change its structure in a matter of hours. Network reorganization events can cause clusters of veins to disappear – a phenomenon also referred to "avalanches" (Marbach et al., 2023b). As mentioned above, network structural properties can change in response to changes in the environment. The behavior of *P. polycephalum* is the result of a self-organized interplay of biochemical and mechanical phenomena that process information, restructure the network, and enable decision-making and foraging. For instance, it has been shown that the global wave pattern created by local oscillations and the network structure both contribute to the dissemination of signaling molecules that indicate the presence of food, which gives the organism the ability to make decisions (Marbach et al., 2016; Alim et al., 2017). However, there is still no clear understanding of which chemicals are involved in this process, and more research is needed to understand why the geometry of the slime mold vein network adapts to the environmental conditions, for instance when it forms direct connections between separated food sources (Nakagaki et al., 2000, 2004).

P. polycephalum can solve mazes: when spread over the entire surface of a labyrinth, a slime mold cell reduces its network to a single vein along the shortest path between two food sources placed at the entrance and exit of the maze. Tubes that do not belong to the direct connections between the two food sources are dynamically deleted during that process. Subsequent studies have increased the complexity of the maze problem. In 2013, Reid and Beekman demonstrated that *P. polycephalum* could find the optimal solution among 32,678 possibilities in an extremely difficult Towers of Hanoi-inspired maze (Reid and Beekman, 2013).

In a subsequent seminal work (Tero et al., 2010), Tero and collaborators showed that *P. polycephalum* connects food sources in such a way that the resulting network has similar cost and efficiency metrics as those of real-world infrastructure networks. For example, when placed on a map of Japan where major cities were covered with food sources, the slime mold formed a network similar to the Japanese railway network. During the morphing process, the veins within the network are strengthened or weakened depending on the amount of fluid that passes through them (Tero et al., 2007). The authors showed that slime molds

Fig. 3. Sketch of a tube in which endoplasmic flow occurs in peristaltic waves of length λ. In slime molds, the typical radius of a tube (a_0) is small compared to λ. Over an elementary length $\delta l \ll \lambda$, the tube is subjected to several forces that tend to dilate or shrink the tube. Marbach et al. (2023a) showed that the tube radius $a(z,t)$ averaged over time $\langle a\rangle(z)$ mainly depends on the radial forces that develop in the tube wall because of the crosslinked filaments inside it that resist the tangential forces that the endoplasmic fluid of viscosity μ applies to the tube wall when it flows with an axial velocity $vz(r,z,t)$. A tangential force induces a shear stress (force per unit area) that is equal to $\mu.\tau$, where τ is a shear rate.

build networks that are both efficient and robust (Tero et al., 2010). The overall length of the networks is reduced thanks to intermediate junctions (Steiner points). The topology of the network results from the combination of coordinated oscillations and the movement of the cytoplasm, allowing the organism to maintain effective connections while eliminating any unnecessary tubes (Tero et al., 2010). Other authors have shown that *P. polycephalum* is capable of solving urban design problems. Slime molds have been used to "redesign" Iberian roadways, creating transport networks that are different from existing ones but with similar performance (Adamatzky and Alonso-Sanz, 2011). *P. polycephalum* has also been used to reroute the M6 motorway through Newcastle (Adamatzky and Jones, 2010). Comparisons between existing anthropically-designed urban networks and their biologically-grown *P. polycephalum* analogs have provided insight into both urban network planning and slime molds performance.

Recently, Fleig and collaborators were able to quantify the contractions of actomyosin-lined tubes, compute cytoplasmic flows and link them to the changes in mass distribution and locomotion (Fleig et al., 2022). They analyzed time-lapse images of idle and feeding slime mold networks and encoded the vein thickness distribution. In their approach, a grey-scale value is assigned to each pixel as a function of its location in the domain: white for a pixel in the substrate, black for a pixel in a very thick vein, and lighter variations of grey for pixels in thinner veins. Each pixel is assigned a value that measures the difference between its grey scale value and the mean of the grey scale values on the entire image - we call that value contrasting index in the following. The pixel contrasting indexes are recorded each time a photograph of the network is taken. A change in the slime mold network results in changes in pixel contrasting indexes. If all the pixels change in the same way between two points in time, for example if all pixels darken to the same amount because of slime mold growth, then no network shape change can be detected with the pixel resolution adopted. This can be understood as two similar behavior modes observed at two different time points. In their study, Fleig et al. (2022) use a Principal Component Analysis (PCA) to identify behavior modes and showed that it is not feasible to discriminate the vein thickness modes, whereas in most biological problems, a few modes dominate the behavior of the organism. Fleig et al.'s temporal analysis showed that a continuous spectrum of modes governs the behavior of slime mold. It was found that large flow rates are achieved when the number of significant modes is small. States in which many modes are active are correlated with unspecific behavior, i.e., transitions of behavior such as change of migration direction. This observation is the basis for Fleig and collaborators' claim that a continuous range of contraction modes enables the network to smoothly transition between a variety of behaviors. Indeed, *P. polycephalum* exhibits throughout its networks of tubes few sustained large-scale oscillations and a continuous spectrum of short-scale oscillations of varying spatiotemporal scale when not faced with any stimuli (Fleig et al., 2022). Adding a stimulus in the environment activates regular large-scale oscillation patterns within this continuous spectrum (Fleig et al., 2022). The authors highlighted that the combination of intracellular signaling and cytoplasm-based fluid dynamics results in collective properties that allow *P. polycephalum* to move and exhibit a complex behavior.

6. Most network models are either non-physical or non-predictive

The remarkable transport efficiency of the vein network in *P. polycephalum* has prompted the development of models to gain insight into the principles at the root of this efficient system. The main models available to date are reviewed in the following. Table 1 summarizes the main features of the models presented.

Probability-based models. Cellular automata models were initially designed to predict the spatial distributions of ant colonies and bee swarms, and were recently extended to slime mold (Hsu et al., 2022).

Table 1
Main features of the slime mold network models available to date.

Model category	Principle	Usage	Limitations
Probability-based	Grid cells change status when an agent occupies it. The agent moves according to probability rules to reproduce aspects of slime mold behavior.	Prediction of optimized network and not only cell shape during feeding and chemotaxis.	Cannot predict the sequence of states between an initial distribution of occupied cells to the optimized network of occupied cells.
Fluid flow analogs	Slime mold initially occupies all the edges of a grid. The edges represent slime mold tubes. An avatar pressure variable is assigned to each node, so as to create higher pressure gradients at the vicinity of food sources. In each tube, diffusion fluid flow controls the tube radius: the more flow, the larger radius. Some tubes eventually disappear from the grid when no flow occurs in them.	Prediction of optimized network connecting food sources and avoiding adverse environments.	Fluid flow in the tube depends on a gradient of pressure that is an avatar for the actual stimuli in the environment (food or adverse chemical substances). The diffusion equation does not represent the diffusion of actual species, but rather a time average of the movement of endoplasm mass. Intermediate network states between the initial state and the final (optimal) one cannot be predicted accurately.
Electronic analogs	Endoplasmic flow is modeled as an electric flux in a network made of charged edges and nodes endowed with different potentials.	Explanation of some aspects of endoplasmic fluid flow and cell migration.	Models are not related to biological variables or principles. Network morphing cannot be predicted.
Particle-agent models	Particles move on a grid according to the direction of chemical concentration gradients, which include concentration gradients of trail markers left by particles at their previously occupied sites.	Can simulate the emergence of persistent dynamic networks, surface minimization and branching. Multi-agent models (e.g., agents biased towards low vs. high trail marker concentration) can simulate self-organization.	Models require evolutionary rules that are often not grounded on biology. Network morphing cannot be predicted.

Cellular automata models assume that a 2D domain is initially covered by shapes that represent the initial state of *P. polycephalum*. Each shape occupies one or more cells of the domain grid and changes geometry when an agent occupies it. The agent moves randomly in the domain covered by *P. polycephalum* and changes the status of the cell that it moves into according to probability rules. Possible cell states include substrate, cytoplasm, and cytoskeleton. The rules are based on the status of the neighboring cells and on the presence of food stimuli that are spread on the domain in the form of discrete sources. The cell automata model proposed by Hsu et al. (2022) can solve traveling salesman problems (to find the shortest possible route that visits a set of given nodes exactly once and returns to the original node) and Steiner tree problems (to find the minimum-length tree that connects a set of specified nodes in an undirected graph), but it cannot predict the

intermediate states of the network between its initial configuration and its final, optimal one. By contrast, stochastic slime mold growth models, inspired by bacterial colony dynamics, were designed to predict network morphing (Wagner et al., 1999). The substrate is represented by a lattice of sites, and the cell is represented by a cluster of occupied sites, as illustrated in Fig. 4A At each step, the algorithm picks one unoccupied site at the periphery of the cell and labels it as "occupied". Wagner and collaborators designed a model in which the probability of selecting a site depends on the age of its nearest occupied sites (Wagner et al., 1999). The age of an occupied site is calculated as a function of the time of invasion and the time at which the stock of nearest nutrients is exhausted. The growth timestep is inversely proportional to the number of sites that can be occupied at the periphery of the cluster, i.e., the growth rate increases over time. The model, verified against experiments in which *P. polycephalum* was grown on a substrate that contained a discrete set of food sources, can inform on the evolution of the spatial distribution of occupied food spots over time, and can highlight the preferential growth at the vicinity of young plasmodium. Later, Takamatsu and collaborators used a stochastic model to explain the effect of chemical gradients on the directionality of slime mold growth (Takamatsu et al., 2009). The grid is made of uniformly distributed sites represented by nodes, and the cell of *P. polycephalum* is represented by occupied nodes and edges. Each node is surrounded by six edges and nodes. The probability of growth to a site adjacent to an occupied node depends on a parameter that controls the direction of growth as a function of adjacent occupied edges and their alignment with neighboring sites that have non-zero concentrations of nutrients and salts. The advantage of this approach is that the model can predict the development of networks, and not just geometric shapes. Overall, stochastic models can calculate a sequence of growth states, but they are grid-dependent and cannot predict slime mold growth patterns in terms of absolute time and length scales. Currently, stochastic models remain restricted to feeding behavior and chemotaxis. Stochastic models might be used to simulate phototaxis and exploration on a non-nutritive substrate, but it is unclear whether they could be applied to predict habituation, because no time variable exists in the formulation, or whether they could accurately simulate cell fusion, because there is no guarantee that the algorithm would converge towards a physical solution.

Fluid flow network analogs. Kobayashi, Nakagaki and Tero led the development of a family of models (called KNT models thereafter) in which laminar flow equations are applied to veins the diameters of which evolve as a function of past fluid mass exchanges (Kobayashi et al., 2006; Nakagaki et al., 2007; Tero et al., 2007). KNT models assume that the network of slime mold covers all the edges of a grid made of tubes in which laminar flow occurs, as illustrated in Fig. 4B The axial fluid flow Q_{ij} in a tube with nodes i and j and length L_{ij} is governed by Hagen-Poiseuille equation, in which flow feedback is accounted for in the tube radius evolution law, as follows:

$$Q_{ij} = \frac{\pi \times (r_{ij})^4 \times (p_i - p_j)}{8 v L_{ij}} = \frac{D_{ij} \times (p_i - p_j)}{L_{ij}}, \frac{dD_{ij}}{dt} = f(|Q_{ij}|) - D_{ij}$$

in which p_i and p_j are the pore pressures at nodes i and j, respectively, r_{ij} is the radius of the tube that joins nodes i and j, v is the viscosity of the fluid and $f(|Q_{ij}|)$ is a decay function (typically a sigmoid) that takes the value 0 when Q_{ij} is zero. The radius of each tube evolves as a function of the probability of flow inside it. Food sources and topological obstacles are represented by imposing concentrated source and sink flows of arbitrary amplitude and by choosing judicious flow feedback parameters (Tero et al., 2010). KNT models can qualitatively forecast polarization and pruning induced by chemical or light gradients. The network dynamics algorithm converges towards the shortest path (Bonifaci et al., 2012; Becker et al., 2019; Karrenbauer et al., 2020). KNT models were successfully applied to road navigation within a network of highways with clogging obstacles (Tero et al., 2006), railroad design (Watanabe et al., 2011) and wireless sensor network optimization (Li et al., 2011).

Later, Meyer and collaborators wrote a stochastic formulation of the KNT model to reflect the effect of lighting spatial variability (Meyer et al., 2017). Models derived from the KNT network algorithm are descriptive but only provide asymptotic estimates of the response of *P. polycephalum* to environmental stimuli, because iterative changes of the lattice do not represent the actual temporal evolution of the slime mold network. Moreover, KNT models cannot predict foraging by shuttle streaming. In fact, flow in the ectoplasm has never been modeled as a peristaltic flow in deformable tubes at the scale of the entire network. An attempt was made to explain post-stimulus network persistence by modeling the diffusion of a chemical agent that softens the tubes of the cytoplasm upon environmental stimulation (Kramar and Alim, 2021). Shuttle flow enlarges the softened tubes where the concentration in chemical agent is high. The proposed model focuses on a single peristaltic tube. Tube wall softening occurs on a timescale that depends on the diffusion kinetics of the chemical agent in the substrate. The model can qualitatively predict the relative change in radius of the network tubes as a function of time in reference to the stimulation time, and as a function of the Euclidean distance to the stimulation point. But the connectivity and tortuosity of the network are not accounted for, which makes it challenging to interpret the persistence of network patterns other than radius enlargement in the neighborhood of nutrients. The model implicitly makes memory encoding dependent on the diffusion coefficient of a chemical agent that has not been identified to date, which means that the diffusion coefficient requires calibration for every experiment. Currently, the parameters that influence the diffusion coefficient, and thus, the persistence span of the observed memory encoding, are still unknown.

Electronic analogs. The flux of calcium and other ions through the membrane of *P. polycephalum* (Achenbach and Weisenseel, 1981; Kuroda and Kuroda, 1981; Fingerle and Gradmann, 1982) triggers the dynamic contraction of the cytoplasm, which exhibits a surface electrical potential (Ridgway and Durham, 1976; Stegemann et al., 1987). The magnitude of that potential varies with the dynamic contractions of the membrane (Meyer and Stockem, 1979). The combination of peristaltic deformation, oscillations in electric charge, and difference in electric potentials throughout the network contribute to the flow of the endoplasm (Kishimoto, 1958). In electronic circuit analogs (see for example Fig. 4C), that flow is modeled as an electric flux in a network made of edges charged negatively or positively, and nodes endowed with different potentials (Adamatzky and Schubert, 2014). Electronic circuit analogs thus help understand the polarity of the flow. Additionally, slime mold was found to transmit electricity between electrodes and an electronic circuit was designed to emulate the signal transmitted by a protoplasmic tube subjected to changes in electric potential (Whiting et al., 2014) or temperature (Walter et al., 2016). An electronic model that encodes the oscillatory internal motion mechanism of slime mold was built with hardware circuits to mimic the expansion of the *P. polycephalum* network and solve mazes (Ntinas et al., 2017). That model was later improved to build slime-mold based memristors (Ntinas et al., 2018). It was also possible to configure the geometry of the *P. polycephalum* network with arbitrary electronic signals to achieve the response of prescribed logical gates (Harding et al., 2018). Recently, electronic analog models were proposed to explain endoplasm flow and cell migration (Oettmeier and Döbereiner, 2019a). Simulations and experiments showed that endoplasmic fluid flow is due to the contraction of fibers at one end of the cell, inducing the periodic contraction of tube segments, known as peristalsis, which in turn initiates fluid transport (Oettmeier and Döbereiner, 2019a). Electronic circuit analogs are not founded on biological principles and do not predict morphing; they shed light on endoplasmic flow, but cannot be used to understand the behavior of slime molds.

Particle-agent models. Particle-like agent models were used to simulate the emergence of optimized transport networks similar to those observed in *P. polycephalum*. In the particle swarm model proposed by Mayne and collaborators, particles orient themselves in the direction of
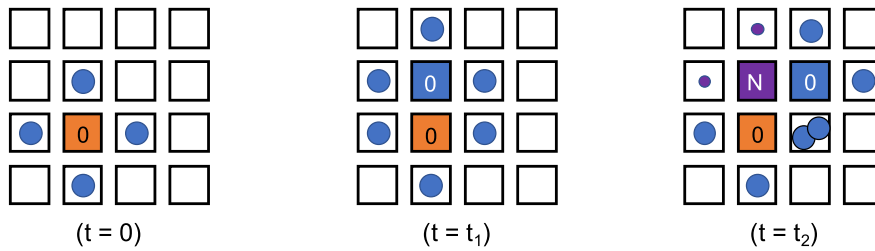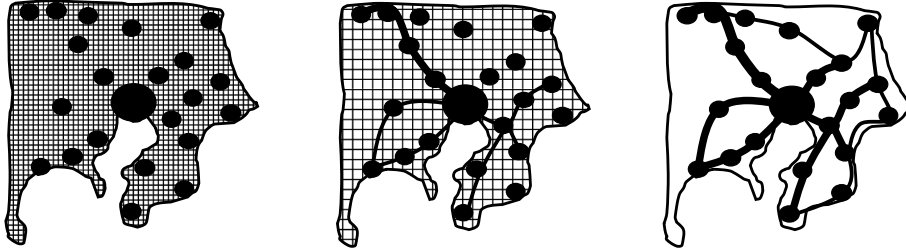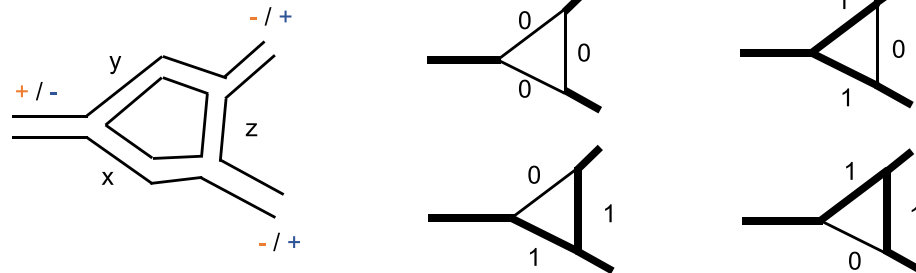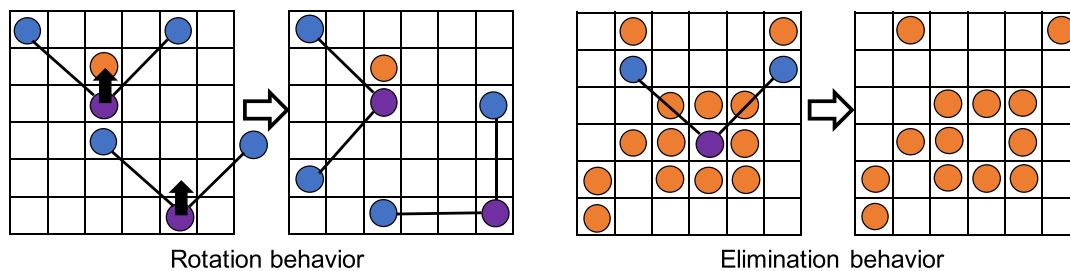
A Stochastic model of slime mold growth

(t = 0)　　　　(t = t$_1$)　　　　(t = t$_2$)

B Fluid flow model of slime mold growth

C Electronic analog

D Particle-agent model

Rotation behavior　　　　Elimination behavior

Fig. 4. Slime mold network modeling techniques. **(A) Stochastic model of slime mold growth** on a heterogeneous model where slime mold can only feed on its initial position (after Wagner et al., 1999). Initially (t = 0), the slime mold can move to any of the four adjacent cells of the grid with equal probability. After one move (t = t$_1$), the slime mold occupies two cells and can move on any of the six adjacent cells with equal probability. The initial cell conserves an age of 0, while the newly occupied cell starts aging from 0 to N at the next move. After two moves (t = t$_2$), the probability of occupancy of the cells adjacent to the aging slime mold cell is lower than the other cells (small dots). One cell can be reached via two different moves and thus has the highest probability of occupancy (two large dots). **(B) Fluid flow model of slime mold growth** (after Tero et al., 2010). Dots represent food sources. The slime mold is deposited on the largest food source. At the initial stage of the calculation, it is assumed that the slime mold forms a dense grid network that connects all the food sources (left). As time passes, edges of the grid disappear if no flow occurs in them and enlarge if large mass transfers occur through them (middle). The slime mold network reaches an asymptotic state in which all the food sources have been reached, and in which the most connected food sources are linked by the wider veins (right). **(C) Electronic analog** for junctions in a slime mold network (after Adamatzky and Schubert, 2014). Tube z is a bypassing channel that is about three times smaller in diameter than tubes x and y. Flow in the junction alternates direction (from + to – and then – to +) every *circa* 55 s. The flow in the junction is similar to that in a XOR gate: no outflow (z = 0, thin line) if x and y both exhibit inflow (x = y = 1, thick lines) or if x and y both exhibit no flow (x = y = 0, thin lines); non-zero outflow (z = 1) if x and y take different values (x = 0 and y = 1 or x = 1 and y = 0). **(D) Particle-agent model** (after Liu et al., 2017). Active agents are represented by purple circles attached to two blue circles that represent the left and right sensors. Inactive agents are represented by orange circles. Active agents rotate (left) instead of moving along the bisector of the left and right sensor directions because they encounter an obstacle (another agent) or move out of the grid. An active agent is eliminated (right) because it can no longer move (blocked by other agents).

the highest chemical gradient and transmit a displacement wave to their neighbors, hence forming chains that represent cytoskeletal filaments (Mayne et al., 2015). Jones proposed a low-level single-agent model in which particles move on a grid in a direction that is dictated by a measure of chemical concentration gradient (Jones, 2010). Each particle deposits a trail marker on the grid where they are located. The marker diffuses and dissipates at rates that are given as part of the input parameters of the model. Each particle measures the intensity of the trail marker with left and right sensors, and a set of conditional rules is applied to decide whether the particle should move forward, left, or right. The length and orientation angle of the step made at every increment of time are also input parameters. Jones was able to simulate the emergence of persistent dynamic networks and to highlight surface minimization and branching behaviors through parametric studies. Liu and collaborators developed a multi-agent model that can mimic foraging and pruning (Liu et al., 2017), as explained in Fig. 4D. Two agent types are deployed and each agent senses trail markers and nutrient markers, which both diffuse and dissipate at rates that are given as part of the input parameters of the model. Agents T1 (respectively, T2) are biased towards areas with low (respectively, high) trail marker concentration, such that agents T1 tend to expand the network, while agents T2 tend to contract it. The initial agents are all T1, and agents T1 transform into T2 when they reach a food source or when one of their neighbors is an agent T2. These evolutionary rules ensure that the model can simulate self-organization. The multi-agent model mimics the growth of *P. polycephalum* in mazes even when the geometry of the maze is changed dynamically. A multi-agent model inspired by Jones's particle-agent model was used to solve the salesman route problem and delivered itineraries that were 20%–50% longer than the optimal ones (Versluis, 2018). Recently, Chance and collaborators used a multi-agent swarm of robots that was able to find food sources and to congregate, hence mimicking foraging and pruning in *P. polycephalum* (Chance et al., 2022). Although multi-agent models can predict optimized network configurations, the evolution of the network from the initial to the optimal configuration typically does not reflect the actual evolution of the biological network, i.e., multi-agent models cannot predict morphing.

To the best of our knowledge, no theoretical framework can properly predict the evolution of the network as it morphs from an initial configuration to a pseudo-asymptotic optimum. Additionally, state-of-the-art bottom-up models rely on assumptions, whereby the evolution of each element of the network is governed by purely empirical phenomenological laws. In contrast, models that couple mass conservation and diffusion equations can successfully represent the growth of the cellular slime mold *Dictyostelium discoideum* in an open domain (Levine and Reynolds, 1991; Höfer and Maini, 1997), but they were not used to predict the evolution of the acellular slime mold *P. polycephalum*, likely because of its large size and the complexities of its network topology, which is absent in *D. discoideum*. State-of-the-art models of *P. polycephalum* do not explain the physical phenomena that drive endoplasmic flow or memory encoding at the scale of the entire network.

7. Perspectives, modeling network and predicting behavior

To date, no theoretical framework can properly predict the evolution of the network as it morphs from an initial configuration to a pseudo-asymptotic optimum or explain the physical phenomena that drive endoplasmic flow or memory encoding at the scale of the entire network.

Developing multi-scale models to update the architecture of a network as a function of local chemical gradients and wave propagation in connected tubes requires collecting datasets in which the evolution of the network of *P. polycephalum* is tracked component by component. A classic way to register the state of a network is to calculate the adjacency matrix of the network, which provides the connectivity of the nodes. However, the adjacency matrix alone does not provide any information

on the geometry of the network, unless the coordinates of the nodes are known. The eigenvalues of the Laplacian matrix, defined as the difference between the matrix of node degrees and the adjacency matrix, were used to encode network features and to derive a predictive model of network evolution from this sequence of features (Wu et al., 2019). This approach, based on the spectral graph theory, was initially proposed to predict the evolution of social networks on the Internet and assumes that the number of nodes is fixed.

Multi-frame object tracking (MOT) holds promise for slime mold network analysis and tracking, especially for networks that not only change in node connectivity, but also experience growth and pruning. MOT relies on the partition of the network into a directed graph at every timestep, and on the calculation of a distance metric between the network components identified at different timesteps. Recognizing a network component from one frame to the next requires a consistent partition of the network over time. Formulating multi-frame object tracking as finding the temporal path of a k-partite graph has been successful for tracking cells (Xie et al., 2009), tips of microtubules (Altinok et al., 2006), and biopolymer network growth and deformation (Xu et al., 2019).

Deep learning expanded the scope and accuracy of MOT across multiple frames (Pal et al., 2021). Deep learning MOT algorithms follow four main steps (Ciaparrone et al., 2020): (i) Object detection and bounding; (ii) Object encoding into latent features; (iii) Calculation of the distance between latent features of objects detected in the current frame and latent features calculated in previously analyzed frames; (iv) Identification of the objects of the current frame with objects detected in previously analyzed frames, based on the distances calculated in step (iii). ID tracker is an example of open access MOT algorithm based on deep learning that was successfully applied to the tracking of individual animal trajectories in collectives (Romero-Ferrero et al., 2019). State-of-the-art deep learning - based MOT algorithms are not directly applicable to tracking *Physarum Polycephalum* networks, in which pseudopods change shape and orientation with shuttle streaming, making it difficult to encode each pseudopod with time-consistent latent features.

But MOT by k-partite graphs holds promise for slime mold network analysis and tracking, because it would allow an automatic detection of network components without resorting to a conversion from pixels to a graph. It remains unclear whether state-of-the-art multi-frame object tracking codes can perform satisfactorily when the input frames vary in quality (e.g., light exposure) or whether those codes could identify sequences of graph states as behavioral events (e.g., pseudopod emergence). Multi-frame network tracking by deep learning has allowed ball tracking and trajectory classification in sports (Huang et al., 2019), and could perhaps bring some solutions to these open issues. Deep learning relies on network component encoding. Sequences of latent features could thus be classified to help characterize the behavior of *P. polycephalum*. Network dynamics could then be the input to a predictive slime mold behavior model based on a probabilistic Markov chain. These modeling hypotheses remain to be tested.

8. Concluding remarks

In this review paper, we have attempted to compile the state of the research on the functions of the slime mold vascular network, network geometric changes in response to external stimuli, and correlations between network changes and adaptive behavior. Perhaps the most intriguing result is that the network changes in response to past experience and could thus encode memory. Despite recent advances, several important aspects of network organization and dynamics remain poorly understood. For instance, while rearrangements in the network topology and morphology in response to environmental stimuli have often been observed and reported in the literature, causal, mechanistic relationships have not been identified yet. As highlighted in this paper, understanding slime mold network morphogenesis and dynamics relies on the

development of powerful models to simulate the development of networks in a flexible environment based on (physical) experimental data. Hence, it is crucial to properly measure biological parameters in order to build models and assess the validity of model predictions. For example, biological tools such as calcium imaging and actin labeling need to be developed for slime molds to quantify the spatiotemporal distribution of the major biochemical components in network dynamics. Measurement of mechanical forces including shear pressure also needs to be extracted to understand flow dynamics and its interaction with the tube network. Lastly, until now, tracking methods to extract slime mold networks have allowed us to describe network topology statically, but these could be improved to understand how the network evolves and responds to environmental changes. This would allow us to better link architecture to function. Outstanding questions that remain unanswered to date include: i) Does the network of slime molds integrate environmental information and act as a repository? ii) Does information stored in the network morphology contribute to adaptive behavior? and iii) Is network adaptation retained as a 'memory' for an extended period of time and does it play a role in subsequent information processing? By dissecting the unique behavior of slime molds at both a biological and physical level, we expect to find some answers to these questions in the near future.

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Declaration of competing interest

The authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest.

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