Biology of the *Physarum polycephalum* Plasmodium: Preliminaries for Unconventional Computing

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Abstract Slime mould *Physarum polycephalum* is a macroscopic amoeba-like organism whose ability to 'compute' the solutions to complex problems ranging from logic to computational geometry has led to its extensive use as an unconventional computing substrate. In slime mould computing devices—'Physarum machines'—data may be imparted to the organism via stimulation with chemical, optical, mechanical or electrical sources and outputs are generally behavioural, chemical or/and electrical. This chapter examines the biological basis of a slime mould's ability to perceive and act upon input data and the mechanisms that contribute towards the output we interpret as computation. Furthermore, various research methods for slime mould cultivation, electrophysiological measurement and hybridisation with exogenous substances are discussed. The data presented here provides an essential foundation for the computer scientist wishing to fabricate their own Physarum machines.

1 Introduction

It has long-since been recognised that the unique physiology of the slime moulds make them ideal research organisms, but the past decade has seen a veritable explosion of research expounding the use of one particular slime mould—*Physarum polycephalum*—as a living unconventional computing substrate, the 'Physarum machine' [1]. Although the foundations of the study of life processes as expressions of 'natural' computing was first formalised in the 1950s by the early cyberneticists, the assertion that a live organism may be utilised in the construction of laboratory prototypes of functional computing systems is nevertheless an unintuitive and esoteric concept at the time of writing. The authors propose that the reason for this is the relative complexity of biological organisms in comparison with computers: we have had the benefit of guiding the evolution of computers from their elementary units and hence have a deep appreciation of how they function. When compared to our

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understanding of biological processes which we have undergone some 3 billion years of intensive but unguided development, it is understandable that whilst we are now programming computers to undertake extremely complex calculations, we are still reading the instruction manual for our own bodies. When we characterise certain natural processes as computation, we are attempting to use biology to perform useful calculations in the manner of a regular computer: it follows, therefore, that a thorough and intuitive understanding of the processes we are hijacking to our own ends is required.

Slime mould is an ideal 'entry-level' biological computing substrate as it is arguably a 'simpler' organism than, say, a mammal. Simplicity is a dangerous word in this context as of course, slime mould is no less developed than any other life form but rather is, by virtue of being a highly resilient macroscopic single cell, somewhat easier to study than organisms composed of a multitude of indivudally fragile and complexly interrelated cells.

This chapter explores the underlying biological phenomena that we choose to characterise in the language of computation and provides the necessary knowledge to begin experimenting in the field of Physarum Computing.

2 Taxonomy, Morphology, Habitat and Life Cycle

Slime moulds are not fungi as their name implies, although they were historically considered to be after their initial taxonomical classification. They are broad, diverse group of amoeboid organisms (phylum *Amoebozoa*, infraphylum *Mycetozoa*) that reproduce via spores and are grouped into three major taxa: the 'true', or 'plasmodial' slime moulds (class *Myxogastria*, but the constituent organisms are more commonly known as *Myxomycetes*), the cellular slime moulds (*Dictyosteliida*) and the often-overlooked *Protostelids* [2, 3]. The former group consists of the slime moulds that exist as a syncytium—a single cell by virtue of the entire organism being encapsulated by a single membrane, but containing more than one nucleus: indeed, a single organism will typically contain many millions of nuclei and may therefore be thought of many cells living in unison, rather than just one single cell [4, 5]. It is for this reason that they were historically called 'acellular', as opposed to 'unicellular', but it is now more common to refer to the true slime moulds by the name of their vegetative (resting) life cycle phase, the 'plasmodium' (*pl.* plasmodia), as this term also implies other facts about the state of the organism. The genus Physarum belongs to this taxon. This is contrasted with the cellular slime moulds, who are composed of macroscopic masses of many distinct cells living in unison, and the Protostelids, who are more distantly-related microscopic variants [2, 6].

The Physarum plasmodium is a yellow amorphous mass (Fig. 2) that can range in size from a few mm^2 to over half a m^2 [7]. The organism will typically be composed of a network of tubular 'vein-like' structures whose topology may dynamically rearrange, which anastamose into a 'fan-like' advancing anterior margin. On nutrient-rich substrates the organism will tend to possess proportionally more fan-like fronts,

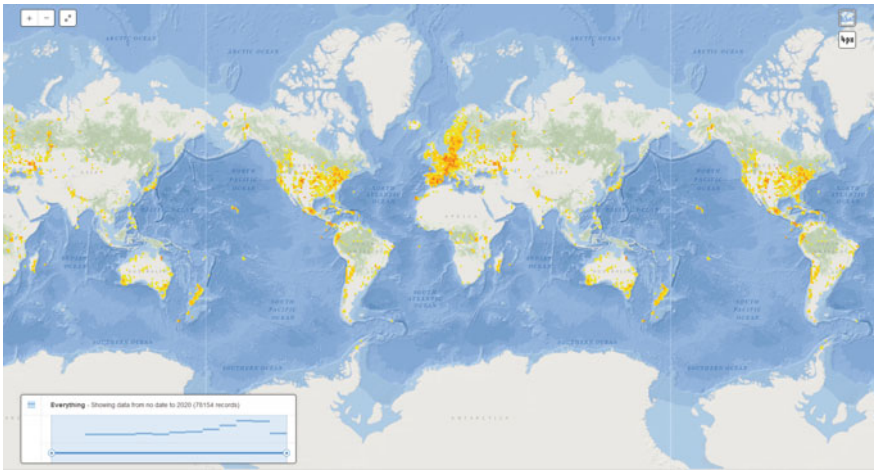

Fig. 1 Map to show prevalence of the genus *Physarum* throughout the world, where *yellow* through *red* marks represent a relative scale of reports of incidence. Reprinted with permission from the Encyclopaedia of Life [10]

implying that these high surface area structures are better adapted for nutrient absorption. The plasmodium is able to crawl at speeds exceeding 1 cm/h; the mechanisms underlying this are explored in Sect. 3.

Physarum and it's related species are found worldwide but are most concentrated in Europe, North America and Japan (Fig. 1) and typically reside in dark, moist

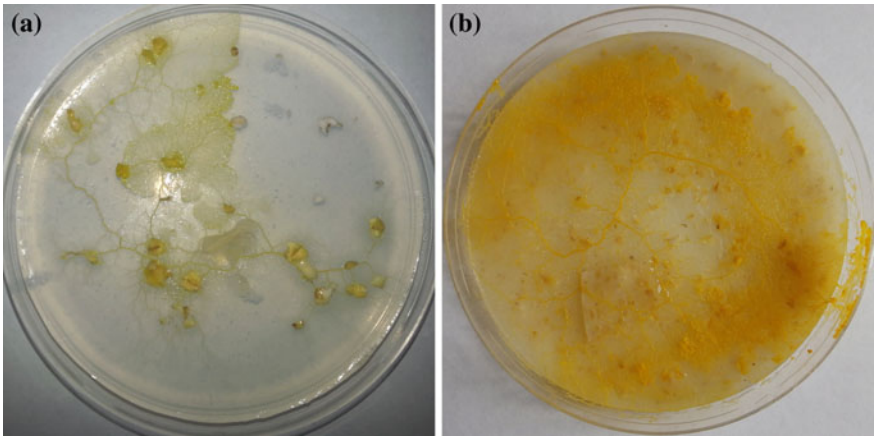

Fig. 2 Photograph of the Physarum plasmodium growing in 9 cm Petri dishes. **a** On non-nutrient agar sprinkled with oats, the plasmodium take a diminutive morphology composed of thin tubular structures and a 'fan-shaped' advancing margin. **b** On nutrient-rich substrates such as agar infused with oat flakes, the organism forms an amorphous mass more akin to the advancing margin in the previous image

places such as the bark of fallen trees when in its plasmodial life cycle stage. Multiple sources state that the organism is both predatory and saprophytic: its natural foodstuffs include fungal spores, bacteria, smaller amoebae and decaying matter, the latter of which may be digested extracellularly through the secretion of enzymes [3, 8]. In laboratory experiments, the preferred nutrient source is ordinary oat flakes, although nutrient agarose (agar) plates are also suitable and fully-defined (axenic) culture media exist [9]. The organism requires a well hydrated substrate. Non-nutrient agar gel or moistened kitchen towel are both widely used experimentally.

All true slime moulds reproduce by sporulation. Certain factors, such as starvation, light irradiation and dehydration will prompt the plasmodium to irreversibly transform into a multitude of black, globulose structures known as sporangia that harbour the organism's spores. Unusually, spores may germinate into either unicellular, uninucleated microscopic amoebae (myxamoebae) or, if the organisms growth

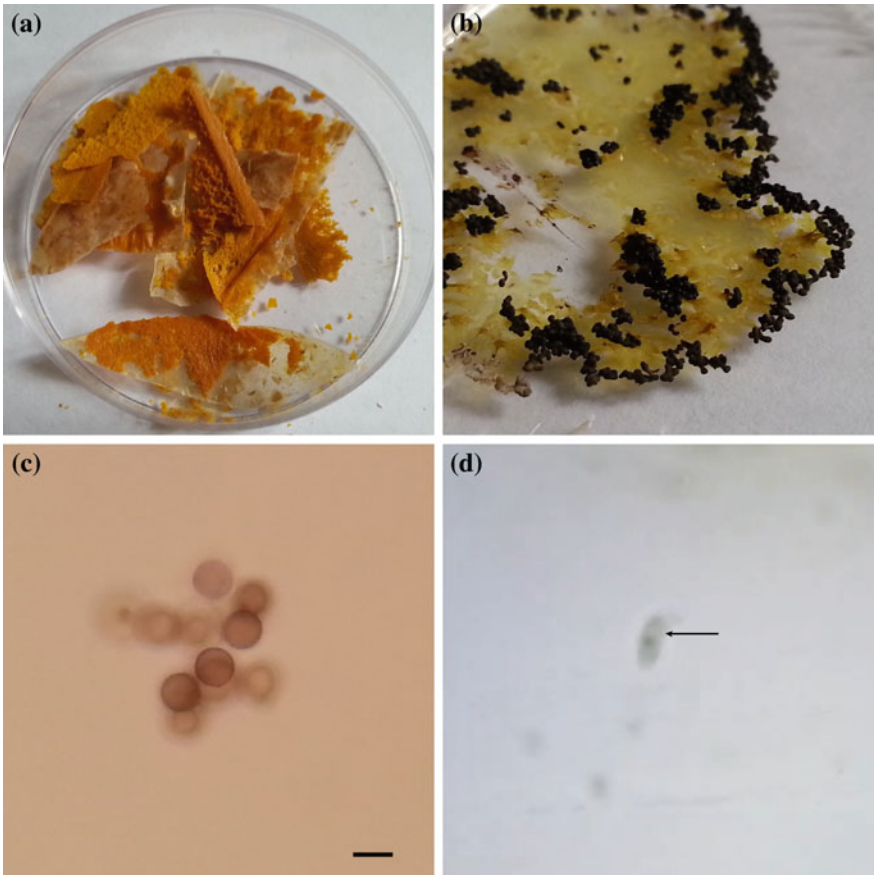

Fig. 3 Photographs to illustrate the life cycle stages of Physarum. **a** Fragments of sclerotium. **b** Sporangia. **c** Spores. **d** Swarm cell (*arrowed*). **c**, **d** Scale bar = 10 μm

medium is liquid, a flagellated version of the myxamoeba known as a swarm cell. Spores, myxamoebae and swarm cells are all haploid—i.e. have half the number of chromosomes of the mature organism—but may reproduce sexually or asexually, as required [3]. Following reproduction, a plasmodium develops. True slime moulds have another life cycle phase called the sclerotium, which is a highly resistant desiccated form that the organism will assume if environmental conditions become too unfavourable. This is a fully reversible process: a sclerotium can be revert back to a viable plasmodium provided suitable culture conditions are provided. As sclerotia remain viable for several years, the slime mould researcher may capitalise upon this to build a long-term stockpile for storage and transport of the organism without the requirement for cryogenic preservation. Some of *P. polycephalum's* life cycle forms are illustrated in Fig. 3.

3 Cell Biology and Physiology

3.1 Motility

The name '*Physarum polycephalum*' is often mis-translated, which is unfortunate as it is very descriptive of the organism. 'Physarum' is commonly quoted as meaning 'slime', but is in fact derived from the Latin *physarion*, which can mean bellows or syringe [11, 12]. Both adequately refer to the rhythmic contraction and relaxation of the organism which drives the movement of fluid through the centre of the organism, as will be explored presently. 'Polycephalum' is less cryptic as it translates fairly directly as 'many headed'. Some have suggested that this refers to its multinuclearity, but this seems unlikely as although the cell nucleus had been observed by the early microscopists in the mid 18th century, it was only fully described in 1803[1] some 9 years after the first description of the genus *Physarum* by Persoon [14]. It seems likely therefore that the name refers to the fact that multiple apparently autonomous leading edges may exist in one plasmodium. This is an observation of note as some of the first work on slime mould computing was based on the principle that Physarum can 'choose' the most efficient path between food sources. The biological basis for this involves the slime mould identifying chemical gradients with multiple advancing margins (or many 'heads') before 'deciding' to navigate along the strongest gradient. This has been interpreted as slime mould undertaking problem solving and network optimization, such as in the ground-breaking experiments that demonstrated Physarum calculating efficient routes through labyrinths and approximating global transport networks [15, 16]. As such, migratory patterns may be thought of as a form of output from a Physarum machine where chemoattractants were used as the input (see Sects. 3.3 and 5).

[1] For the reader's interest, Brown is credited with the discovery which was presented in 1831, but he graciously acknowledged the earlier observations of Bauer [13].

Physarum achieves motility by rhythmic propulsion of its cytoplasm via the contraction of muscle proteins that sit circumferentially about the interior of plasmodial tubes. Cytoplasm flow oscillates anteroposteriorly every 60–120 s. Net anterograde movement is achieved by gelation of the posterior end and solation of the anterior margin, combined with tip growth of intracellular protein networks [7, 17, 18]. These protein networks, which are collectively known as the cytoskeleton, are predominantly composed of actin, which provides mechanical support, a network for intracellular signalling and participates in the muscular contractions which propel the cytoplasm with the aid off another muscle protein, myosin [19]. This regular contraction-relaxation cycle that propels the cytoplasm is known as shuttle streaming, which also serves to distribute the contents of the cytoplasm (organelles, absorbed foodstuffs etc.) throughout the organism. It has been suggested that the plasmodial actin network is a rich medium for over-riding natural signalling processes to implement intracellular computation [20].

New evidence has surfaced in recent years indicating that contractions in networks of protoplasmic tubes are peristaltic, i.e. discrete waves of contraction propagate through the tube network according to a contraction pattern consisting of a single wavelength [21]. Although oscillators with apparently simple dynamics are an attractive substrate for unconventional computing, the researcher should be aware that historical literature emphatically states that tube contraction is simultaneous and monorhythmic in larger tubes, at least in smaller plasmodia [7, 17].[2]

3.2 Cytology

The plasmodial tube may be sub-divided into three distinct layers (Fig. 4):

1. The slime layer (glycocalyx). This is a sheath of mucopolysaccharide-rich liquid coating the organism after which it is named [7]. Its purposes are many, but include protecting the organism from desiccation and aiding the solubilisation and extracellular digestion of food. The slime layer also mediates one of the most interesting characteristics of the organism, which is the extracellular spatial 'memory' that was first described relatively recently by Reid et al. [23]. As a plasmodium migrates around its environment, it leaves an trail of slime in its wake which contains the organism's effluvia. The plasmodium is able to sense the chemicals it leaves behind and will avoid areas it has previously visited by virtue of this mechanism: the aforementioned researchers found that this extracellular 'memory' is used by the organism to allow it to solve problems of navigation that are usually reserved for robots, such as the U-shaped barrier test.
2. The ectoplasm. This is a highly vacuolated region which sits circumferentially about the tube periphery and contains the majority of the organism's cytoskeleton: it's actin network, in particular, is extremely dense here and is oriented in radial,

[2]It is beyond the scope of this chapter to discuss the discrepancies between recent and historical results but we refer the reader to Refs. [7, 21, 22] for a deeper explanation.

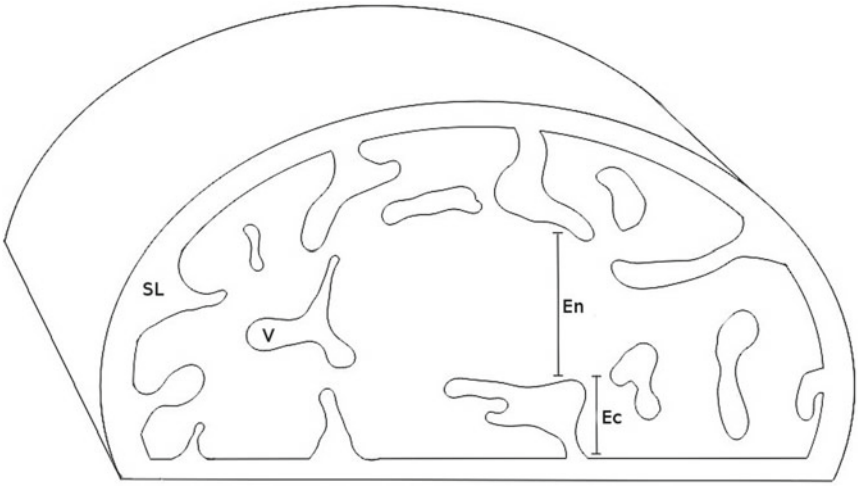

Fig. 4 Schematic diagram of a transverse section through a plasmodial tube. SL: slime layer, V: vacuole, Ec: ectoplasm, En: endoplasm. Adapted from Ref. [28]

longitudinal and spiral patterns (Fig. 5b) [24–26]. Cytoplasm flows extremely slowly through this region and hence it is often referred to as a gel when characterising the organism as a gel-sol system.

3. The endoplasm. This region comprises the hydrodynamic core of the organism through which the cytoplasm moves rapidly. It is the sol component of the sol-gel system. Although the boundary between ecto- and endoplasm is indistinct, it is readily distinguishable by its comparative lack of vacuoles. When muscle proteins contract in the ectoplasm, it generates pressure in the endoplasm, thereby producing propulsive force [27].

As aforementioned, plasmodia contain a great many nuclei, the number of which can exceed 10^8 per organism [29]. The majority of a plasmodium's nuclei are concentrated at the anterior margin, presumably as it is the most metabolically-active region, but plasmodial tubes also contain a reasonable number of nuclei within the endoplasm (Fig. 5). It seems likely that nuclei are anchored to and transported upon the organism's actin network [20]. As a eukaryotic cell, slime moulds contain most of the organelles that one would expect to find in a mammalian cell, including golgi apparatus, mitochondria and endoplasmic reticulum (Fig. 6). Unlike human cells, however, the plasmodium contains numerous phospholipid membrane-bound vesicles whose purposes include endocytosis, exocytosis and transcytosis (see Sect. 4).

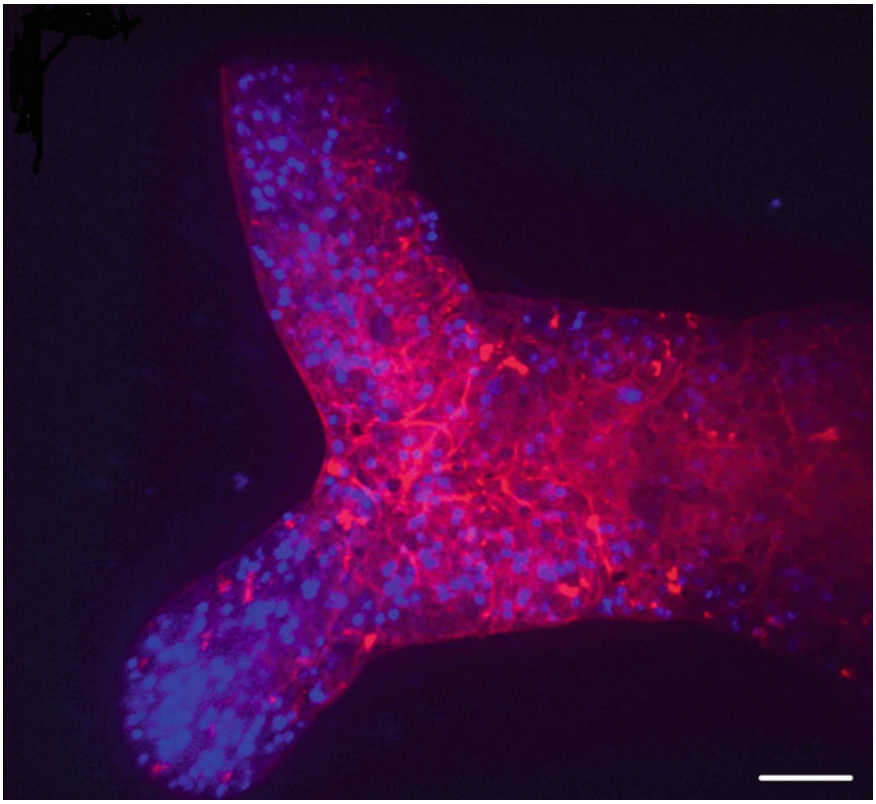

Fig. 5 Confocal micrograph showing nuclei (*blue*) and actin (*red*) in a 4 μm-thick transverse section through a plasmodial tube. Nuclei are more concentrated in the growing tip in the *lower-left hand* portion of the image and the actin network is extremely dense throughout. Scale bar = 40 μm. For methods, see Ref. [20]

3.3 Bioelectrical Characteristics and Chemical Oscillators

Slime mould computing is an art of 'programming' the organism with inputs it can perceive (which is covered in Sect. 5) and interpreting some aspect of the organism's behaviour as an output [30]. When choosing the most suitable output to measure, one must consider aspects such as speed of response, repeatability and relative complexity of the interface. Measurement of bioelectrical activity is an attractive prospect as electrical responses to stimulation are easy to measure and relatively rapid when compared to interpretation of migratory behaviour. Electrical measurements may also be automated and interfaced with conventional hardware with relative ease.

The simplest way to achieve this is to measure potential non-invasively through a thin layer of agar via underlying electrodes (see Sect. 6 for details of methods). When measured in this manner, plasmodial bioelectricity oscillates rhythmically with a typical amplitude of 1–15 mV. This electrical oscillation directly corresponds with the

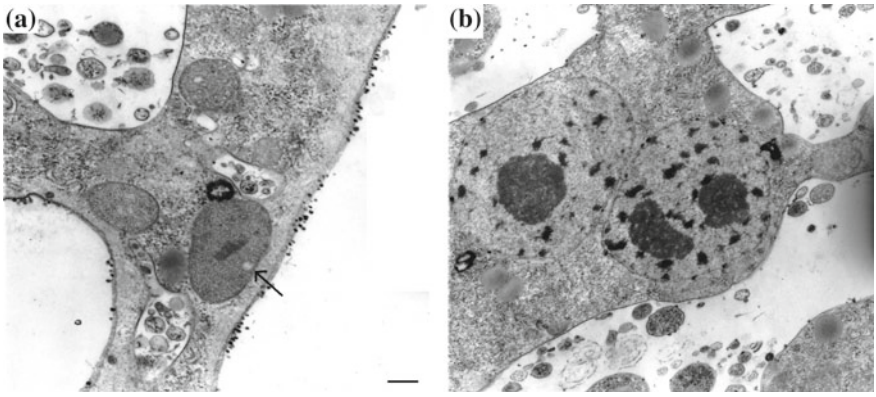

Fig. 6 Transmission electron micrographs of 80 nm ultrathin transverse sections through a plasmodial tube, demonstrating the presence of several organelles in the ectoplasm. **a** The outer membrane of the ectoplasm: three mitochondria are present, one of which is *arrowed*. Endocytotic vesicles are also present. **b** Two nuclei in different replictory stages are adjacent to several vesicles. **a**, **b** Scale bar = 1 μm. See Ref. [28] for methods

period of shuttle streaming: the cytoplasm reverses at each peak and trough. This can be investigated by concurrently observing the organism via light microscope whilst measuring electrical activity: tube diameter oscillates in time with electrical signals and cytoplasm flows, which is presumably a result of the muscular contractions in the ectoplasm that drive shuttle streaming (Fig. 7). Tube electrical resistance also oscillates rhythmically with the same period as shuttle streaming [28].

The key determinant of plasmodial electrical activity was once thought to be the influx/efflux of calcium ions corresponding with actomyosin contraction/relaxation, but this has been repeatedly called into question. It is now thought that membrane potential is driven by hydrogen ion pumps which reflect the state of the organism's metabolic oscillators [31, 32].

The researcher should be aware that the plasmodium cannot be innervated electrically (although it does exhibit mild galvanotaxis, see Sect. 5) and that multiple chemical and biophysical oscillators contribute to the periodic events we may observe, the most notable being levels of ATP (a biological molecule that may be regarded as 'energy currency'), macromolecular synthesis, enzyme synthesis and intratubular pressure. We refer the reader Ref. [7] for a review of the historical literature concerning plasmodial chemical oscillators with reference to motive systems.

4 Plasmodial Incorporation of Exogenous Materials

The ability of the Physarum plasmodium to internalise and retain exogenous substances was first described in 1960 by Guttes and Guttes [33] who distinguished that some, but not all, of the numerous vesicles that travel through the plasmodium are

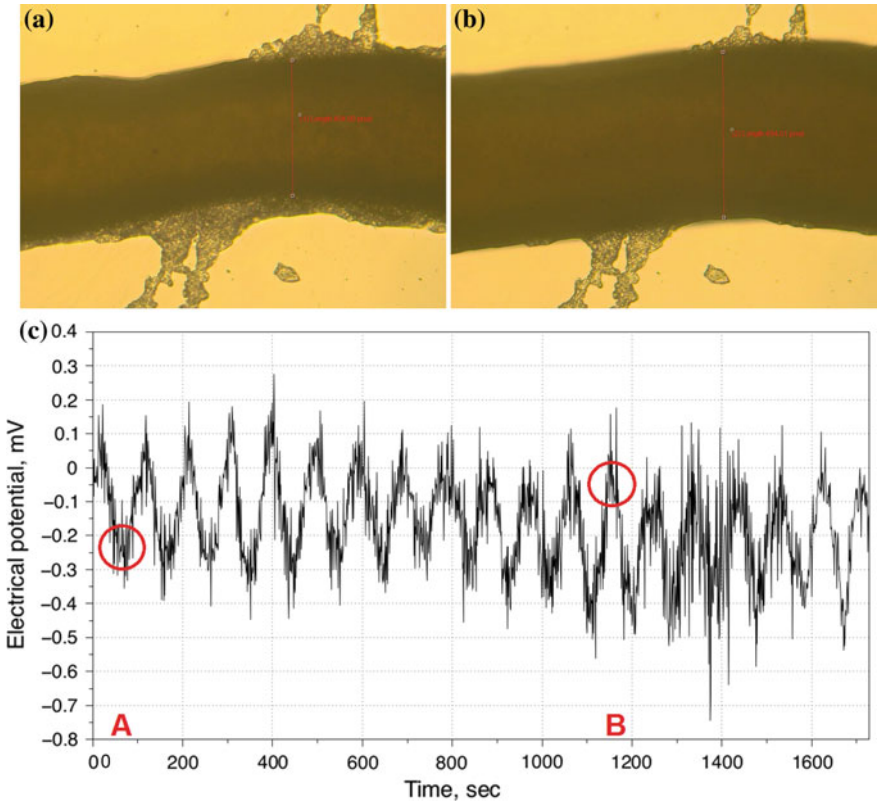

Fig. 7 Correlative light microscopy and electrophysiological measurement of a plasmodial tube. **a**, **b** Photomicrographs of a plasmodial tube taken approximately 1100 s apart with diameter measurements. The tube dilates to nearly 125 % of its minimum diameter. **c** Graph of membrane potential showing the time points at which the photomicrographs were captured (*red circles* and text)

pinocytotic (involved in the internalisation of small objects and fluid). It was not until the sudden surge of interest in slime mould as an unconventional computing substrate, however, that it was realised that this mechanism could be manipulated for practical purposes: studies by Nakagaki et al. [34] and Adamatzky [35] reported that, through the saturation of nutrient sources in coloured food-grade dyes, Physarum plasmodia can be 'fed' substances of interest. Coloured compounds were found to not only discolour the entire organism following 'feeding', but were retained to such a degree that if a second, different-coloured 'meal' was provided, both dyes become mixed in situ to produce an appropriate secondary colour; this was used to indicate the successful implementation of programmable colour-mixing operations in the latter reference. Feeding is therefore a potential mechanism by which exogenous substances of interest may be introduced into the organism which is a viable route to

achieve plasmodial hybridisation, e.g. with artificial circuit elements [28, 36], and is explored more thoroughly in Chap. 7.

The basis for plasmodial integration of environmentally-acquired material include both pinocytosis and phagocytosis [37], however, the latter being a mechanism not dissimilar to those employed by leukocytes for removing pathogens from the bodies of mammals. Both forms of substance internalisation are collectively known as endocytosis. Where pinocytosis involves the in-folding of the cell's membrane to make 'pockets' that catch small objects from the environment that are consequently internalised within phospholipid membrane-bound vesicles, phagocytosis is the extension of finger-like projections (pseudopodia) from the cell's membrane through the momentary assembly of protein scaffolding at its peripheral regions which form invaginations around the item to be internalised, which are typically far larger than those that are pinocytosed. Eventually, the tips of extending pseudopodia fully engulf the foreign substance which then diffuses into the cell in a vesicle. Crucially, whilst slime mould endocytotic mechanisms are not well-characterised, the internalisation route may alter the way in which the endocytosed material is mixed with the cell's cytoplasm.

Does this imply, then, that we are functionally limited to the size of object we can coax into the organism, and if so, does this limit the usefulness of the 'feeding' technique? Will some objects be internalised whilst others are transcytosed? Indeed, Githens and Karnovsky [38] suggested that the optimum size of object that the cellular slime mould *Polysphondylium pallidum* can internalise is about 1 μm, although the differences between the cellular slime moulds and acellular varieties such as Physarum disallow direct comparison. In any eventuality, if Physarum is indeed able to internalise a range of sizes of exogenous object, it seems likely that different sized objects will be internalised by different mechanisms which may in turn alter the ways in which they interact with the organism.

5 Programming the Plasmodium: Attractants and Repellents

To program a Physarum machine we must deliver it 'information' in a format that it can understand and interpret [30]. Furthermore, if we are to interpret the resulting phenomena correctly, we must be able to measure a repeatable and unambiguous output, just as we would have to when designing a conventional computer. This section briefly delineates each of the input types we may use with slime mould computing devices, outlines their underlying biological bases and discusses their benefits and detriments.

5.1 Chemical

As previously discussed, a number of slime mould computing devices fabricated to date rely heavily on *P. polycephalum's* ability to sense and migrate towards or away from certain chemical gradients (chemotaxis). As this is essentially interpreting the plasmodium's inherent foraging behaviour as a 'useful' output, we can state that the organism is being 'programmed' with strategically-placed food sources.

The plasmodium is sensitive to a wide variety of chemicals, however, not all of which are attractants. Slime mould is attracted to compounds known to sedate mammals such as valerian root, suggesting that phenomena such as reception of pheromonal and sedative chemicals in higher organisms may have roots in single-celled life [39, 40]. In a comprehensive study, de Lacy Costello and Adamatzky [41] assessed the relative strengths of plasmodial attraction and repulsion from a wide range of volatile organic compounds, in which it was found that the plasmodium displays a strong preference for non-oxygenated terpene derivatives and repulsion from compounds such as alcohols and aldehydes. Whilst it is perhaps not surprising that some of the compounds have the effects they do (e.g. repulsion from aldehydes due to their being potent biocides), these studies demonstrate that the degree of repulsion or attraction of a chemical may be tightly controlled by appropriate choice of compound and concentration. Plasmodial behaviour has been accurately modelled with multi-agent modelling developed by Jones [42], wherein individual particles follow simulated attractant and repellent gradient.

At the molecular level, chemotaxis involves stimulation of the plasma membrane, through which a signal is transduced to the organism's motive system. Some chemical signals are received by membrane-bound chemoreceptors which cause intracellular chemical signals to be generated to a degree proportional to the amount of receptors activated and the strength with which the sensed compound binds with the receptors. Receptors may have an excitatory or inhibitory effect, corresponding to the generation of attractive and repulsive behaviour, respectively. Chemicals for which the organism doesn't have receptors interact with the membrane electrostatically or diffuse through it, which will also lead to the generation of an appropriate intracellular chemical signal [43]. Attractive chemical signals precipitate the activation of a variety of systems coordinated by second messenger pathways which promote local cytoskeletal assembly (leading to tip growth), ectoplasmic solation and acto-myosin contraction; *vice versa* occurs with chemorepellents [44].

It is clear that chemicals are a powerful form of input into the Physarum machine, but their use is not without detriments. The experimental use of attraction/repulsion as input usually implies that plasmodial migration will be the result of any computation, which is, as aforementioned, a slow process. Furthermore, as it is very difficult to prevent chemical signals from diffusing into substrates such as agar, it is virtually impossible to implement a 'dynamic' chemical input that can be removed mid-experiment.

5.2 Optical

Physarum is an inherently photophobic organism and will tend to inhabit shaded areas in its natural habitat, presumably to avoid dehydration and ultraviolet light-induced cellular damage. Laboratory experiments should ideally be conducted in a complete absence of light, although low-intensity white light or wavelengths in the infra-red region are acceptable if the organism is irradiated continually. Different wavelengths of light can precipitate profoundly different behaviours, although published data is sometimes contradictory concerning their exact effects: ultraviolet and blue light are cited as promoting sporulation but is also strongly repellent, whereas red light has been reported to be an attractant [45, 46]. In more recent studies, green light generated by LED arrays was found to be an adequately strong repellent without causing noticeable deleterious health effects (when compared with blue, yellow and red, Fig. 8), although light intensity is the overriding factor when comparing sources of different intensities.

Cytoplasmic photoreceptors are responsible for absorbing light and transducing the stimulus, the absorption spectra of which peak at 370 nm [45, 48]. These receptors are pigment molecules which undergo a conformational or chemical change following the absorption of a photon within a particular range of wavelengths which then proceeds to assume a high energy state and catalyses a signal transduction cascade. This is similar to the manner in which chlorophyll contributes to the conversion of sunlight to chemical energy in plant cell chloroplasts. When choosing a wavelength of light to use as a stimulus, one must be aware that although UV light induces sporulation (which is irreversible) at low intensities and plasmodial death

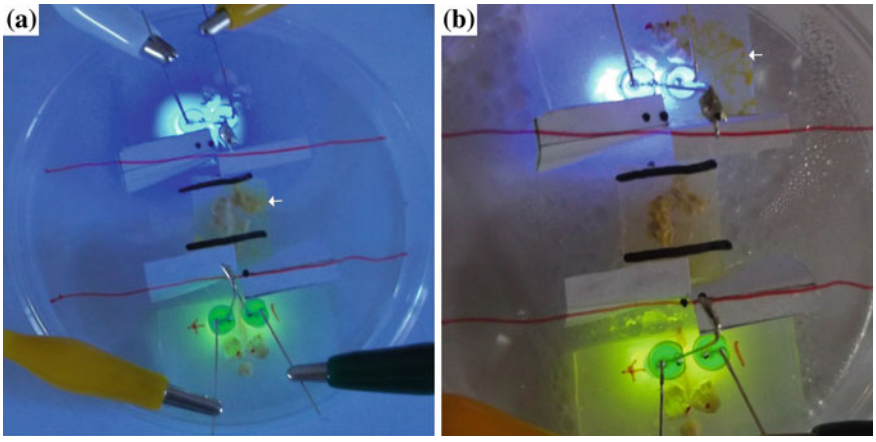

Fig. 8 Photographs of an experiment designed to determine the most repellent colour of LED, wherein the plasmodium (*arrowed*) is given a binary choice to migrate between two nutrient sources illuminated with different colours of light. See Ref. [47] for full methods and results. **a** Time = 0 h. **b** Time = 12 h. The plasmodium has choosen to migrate towards *blue*

at higher intensities, 650–670 nm light has also been described as causing sporulation, although green light (even in the presence of other wavelengths) between 540–620 nm may prevent sporulation [45, 49]. Stimulation with other wavelengths or mixed-wavelength sources of high-enough intensity will precipitate sclerotinization.

As an input into a Physarum machine, light has the benefit of being dynamic, i.e. it can be activated and deactivated during the course of an experiment [50]. Functional slime mould computing devices fabricated to date include systems mimicking reaction-diffusion wave dynamics [51] and logical gates (see Sect. 4). The plasmodium has been reported as responding to certain wavelengths of light with alterations in electrical potential waveform; whilst this suggests that light is an ideal coupling mechanism for an automated computer interface that concurrently interprets plasmodial bioelectrical phenomena, its electrical responses are quite variable and hence the usefulness of this approach is limited [52].

If measuring plasmodial behaviour in response to optical input as a form of computational output, the researcher should be aware that many of the commonly used methods for visualising certain aspects of the system will destabilise it, e.g. exposure to a powerful fluorescent lamp or laser in an epifluorescence/confocal microscope will likely have effects on local electrical and contractile systems, even if exposure is only brief. This can be particularly detrimental if studying phenomena such as streaming velocity or substance uptake (a common situation that is only rarely compensated for!).

5.3 Tactile

The Physarum plasmodium is sensitive to the application of mechanical pressure and has been demonstrated to function as a tactile sensor in both human-interpreted and computer-automated devices [53, 54] and tactile stimulation-induced alterations to cytoplasm flow rate through tubes have been used as an input in microfluidic logic gates [55] .

The mechanisms underlying thigmoreception are likely related to stretch-mediated induction of the calcium oscillator as discussed in Sect. 3.3. Application of gentle tactile pressure—such as ‘prodding’ a plasmodium with a hair or laying a thin glass capillary tube across it—causes a repeatable, characteristical spike in membrane potential which will tend to recede into normal oscillation after 30–40 s; during this time, the organism appears to enter a refractory phase where it cannot be re-stimulated to induce the same effect [54]. Repeated or/and high-intensity stimulation on a single point will cause the organism to migrate away.

Tactile stimulation will cause an area of plasmodium surrounding the point of stimulation to gelate and cease streaming for a period of time proportional to the intensity of the stimulus and inversely proportional to the robustness of the point being stimulated, i.e. a thick tube or fan-shaped margin will only cease streaming

for a short time compared to a thin tube stimulated in the same manner. This can cause severe difficulties with experimental techniques such as microinjection (see Sect. 6.4).

5.4 Other Stimulants

Physarum plasmodia have been reported as migrating towards the cathode in the presence of a DC field (galvanotaxis) and hence this may be used as a Physarum machine input to guide migration, particularly as plasmodia with previous exposure will always migrate towards an electrical field [56, 57]. The organism appears to lack a specific behavioural response when directly electrocuted,[3] indeed it seems to be very tolerant to such abuse, although deleterious health effects occur with higher voltage/current sources. This tolerance to electrical stimulation has led to the use of slime mould as discrete circuit elements, such as electrical wires and bandpass filters [59, 60]. The electrical resistance of a single 10 mm plasmodial tube is approximately 10 MΩ, although this value oscillates by several MΩ as the organism streams.

Following the discussion of light and electrical fields, it is pertinent to note the plasmodial responses to other forms of radiation. Physarum has recently been described as magnetotactic, implying that this is yet another mechanism for controlling plasmodial migration despite the underlying mechanism being unclear [61]. Extremely low-frequency electromagnetic radiation appears to retard streaming [62].

6 Appendix: Research Methods

6.1 Plasmodium Cultivation

Plasmodia may be cultivated on a wide variety of substrates, but to guarantee rapid growth and minimise the risk of microbial contamination, the authors favour using 2 % non-nutrient agar gel in 9 cm plastic Petri dishes or unpatterned kitchen towel moistened with a few millilitres of deionised water in a lidded plastic box. Standard porridge oats should be provided as the sole nutrient source and propagating colonies should be kept in the absence of light at room temperature. Subculturing should be performed every 3–4 days, or when the organism has colonised the majority of its environment, which can be performed by cutting a section of the plasmodium's substrate and transferring it to a new dish or homogenising the organism with a spatula

[3] Parenthetically, it is interesting to note that following the studies that pioneered slime mould—and indeed, human—electrophysiological measurements in the 1950s, researchers were surprised to note that the organism could not be electrically innervated in the same manner as neurons [58], although we now know this to result from *P. polycephalum's* electrical oscillator being controlled principally by chemical factors.

or scalpel blade and transferring it to a new environment. Homogenisation does not appear to significantly alter growth rate as the organism will coalesce within 1–2 h, although growth rate will be severely reduced if the homogenate contains relatively few nuclei: as such, homogenates should always be taken from the advancing anterior margin if growth speed is an important factor.

If working in sterile conditions, various nutrient agar varieties may be used, as can several types of liquid medium [63], although both will induce plasmodial morphologies that are radically different from the type that is observed in nature. Such preparation methods are not commonly used in slime mould computing devices as, aside from the additional complications of maintaining sterility in laboratories that are not necessarily equipped for microbiology, there has been a general trend of utilising the organism's natural form for computing as the wild-type morphology is presumably better adapted for survival. For this reason, preparation of other plasmodial derivatives such as thin-spread plasmodia, microplasmodia and spherules are not discussed here, but further details may be found in Refs. [64–67].

All plasmodia utilised in the experimental studies detailed in this anthology were strain HU554 × HU560, unless otherwise stated.

6.2 Preparation and Revivification of Sclerotia

Sclerotinization can be initiated by gradually dehydrating a plasmodium in the absence of other unfavourable environmental factors. The authors' preference is to transfer plasmodial homogenate to a moistened filter paper in a vented box. This process usually takes about two days. Sclerotinization may also be achieved by dehydrating agar plates (both nutrient and non-nutrient) in fume cupboards, but the researcher should be aware that this carries a greater risk of microbial contamination, particularly by fungi.

Reviving sclerotia is extremely simple and is achieved by placing a piece of sclerotium in a moist, dark environment, such as those detailed in Sect. 6.1. The rate at which the organism revives is surprisingly rapid.

6.3 Measurement of Membrane Potential

Unless otherwise stated, all electrical measurements performed in this anthology were standardised to the following entirely non-invasive specifications to allow for direct comparability. Two 90×10 mm aluminium tape electrodes are stuck to a clear, non-conductive surface such as a glass microscope slide or the base of a Petri dish. A 10 mm gap separates the two and each has a 0.25–0.50 ml hemisphere of 2 % non-nutrient agar prepared at the tip. A plasmodial homogenate or cutting is placed on one hemisphere and an oat flake on the other. The environment is then sealed (usually inside a Petri dish bonded with paraffin film) and left in the dark at room

temperature to propagate. Within 12–48 h, the plasmodium propagates across the gap between the electrodes, forming a single tube between the two: as such, any subsequent electrical measurements will be from a single 10 mm tube. Depending on local environmental factors, some optimization of this set-up may be required if plasmodia fail to propagate; the organism is reluctant to traverse dry surfaces if it finds its environment unfavourable.

Voltage and resistance measurements are performed with the researcher's choice of data logger/multimeter. It should be noted that in such an arrangement, the electrical properties of the organism are being effectively measured through two resistors, i.e. the agar hemispheres, which were found in Ref. [28] to have an average resistance of about 20 KΩ. Because of this, certain results (e.g. oscillating potential amplitude) will be significantly different to other sources who use more conventional electrophysiological testing procedures.

Intracellular measurements are possible but problematic to implement. This is because the organism will tend to seal puncture wounds caused by intracellular electrodes and migrate away rapidly. Consequently, slime mould electrophysiological measurements should necessarily be ingenious and minimally invasive, such as the moist chamber method demonstrated historically by Iwamura [68].

6.4 Microinjection

In instances where one is required to load the plasmodium with a substance and feeding/endocytosis isn't a viable route, microinjection is a workable alternative. A relevant example of such an instance is loading the plasmodium with fluorescent compounds for live-organism microscopy: the dye may have a short half-life, be metabolised too rapidly or may simply not be endocytosed due to its toxicity.

As aforementioned, plasmodial microinjection is unfortunately problematic. This is in part due to its propensity to gelate and cease streaming following vessel injury, but also due to the practical considerations of injecting a very fine-tipped needle into a comparatively enormous vessel whose internal fluid pressure is constantly fluctuating. Indeed, major plasmodial tubes are so thick that even when the organism is cultivated on a sub-1 mm layer of agar overlying a thin glass microscope coverslip and visualised with an inverted microscope, there is still insufficient depth of field to adequately view the tube. Automated microinjection systems should be avoided as the quantities of fluid required to be injected are extremely high compared to the femtolitre requirements of individual cells.

Microinjection should be performed with a sub-10 μm needle injected at an acute angle (relative to the direction of cytoplasmic flow). The needle should ideally be orientated laterally with respect to the tube. Finally, injection should be as rapid as possible, as gelation will still occur even after taking these precautions [44]. This has been successfully used to replace the endoplasm with artificial media and introduce fluorescent calcium dyes into live plasmodia and microplasmodia [32, 69, 70].

References

1. Adamatzky, A.: Die Naturwissenschaften **94**(12), 975 (2007). doi:10.1007/s00114-007-0276-5. http://www.ncbi.nlm.nih.gov/pubmed/17603779
2. Fiore-Donno, A.M., Nikolaev, S.I., Nelson, M., Pawlowski, J., Cavalier-Smith, T., Baldauf, S.L.: Protist **161**(1), 55 (2010). doi:10.1016/j.protis.2009.05.002
3. Stephenson, S., Stempenson, H.: Myxomycetes: A handbook of slime moulds. Timber Press, Portland, Oregon, USA (1994)
4. Gray, W., Alexopoulos, C.: Biology of the Myxomycetes. Ronald Press, New York, USA (1968)
5. Alexopoulos, C.: The Biology of Physarum and Didymium. Academic Press (1982), Cell Biology: A series of monographs, vol. 1, chap. 1: Morphology, Taxonomy and Phylogeny
6. Olive, L.: Bot. Rev. **36**, 59 (1970)
7. Alexopoulos, C.: The Biology of Physarum and Didymium. Academic Press (1982), Cell Biology: A series of monographs, vol. 1, chap. 5: Plasmodial structure and motility
8. Dussutour, A., Latty, T., Beekman, M., Simpson, S.J.: Proc. Natl. Acad. Sci. USA **107**(10), 4607 (2010). doi:10.1073/pnas.0912198107
9. Goodman, E.: J. Bacteriol. **111**, 242 (1972)
10. E. of Life. Physarales species distribution (2015). http://eol.org/pages/5756/maps. Accessed 08 July 2015
11. M.W. Dictionary. Physarum definition. http://www.merriam-webster.com/dictionary/physarum (2015)
12. Geroulanos, S., Panaretos, C., Lyberopoulou, E.: Medicine and Healing in the Ancient Mediterranean. Oxbow Books (2014), chap. 20: Surgery in Byzantium
13. Wayne, R.: Plant Cell Biology. Elsevier (2009), chap. 16: The nucleus
14. Persoon, C.: Neues Magazin fur die Botanik **1**, 63 (1794)
15. Adamatzky, A., Jones, J.: On electrical correlates of Physarum polycephalum spatial activity: can we see Physarum Machine in the dark? Biophys. Rev. Lett. 6(01n02), 29–57 (2011)
16. Nakagaki, T., Yamada, H., Tóth, A.: Biophys. Chem. **92**(1–2), 47 (2001). http://www.ncbi.nlm.nih.gov/pubmed/11527578
17. Grebecki, A., Cieślawska, M.: Cytobiologie **17**, 335 (1978)
18. Cieślawska, M., Grebecki, A.: Current research on Physarum, vol. 120 (1979), chap. Synchronal pulsation in plasmodia of Physarum polycephalum, pp. 167–170
19. Janmey, P.: Physiol. Rev. **78**(3), 763 (1998)
20. Mayne, R., Adamatzky, A., Jones, J.: Communicative Integr. Biol. **7**(1), e32097 (2014)
21. Alim, K., Amselem, G., Brenner, M.P., Pringle, A.: Proc. Natl. Acad. Sci. **110**(33), 13306 (2013). doi:10.1073/pnas.1305049110/-/DCSupplemental.www.pnas.org/cgi/doi/10.1073/pnas.1305049110
22. Tyson, J.: The Biology of Physarum and Didymium. Academic Press (1982), Cell Biology: A series of monographs, vol. 1, chap. 3: Periodic phenomena in Physarum
23. Reid, C.R., Latty, T., Dussutour, A., Beekman, M.: Proc. Natl. Acad. Sci. USA **109**(43), 17490 (2012). doi:10.1073/pnas.1215037109
24. Rhea, R.: J. Ultrastruct. Res. **15**, 349 (1966)
25. Olenhusen, K., Wohlfarth-Bottermann, K.: Cell Tissue Res. **196**, 455 (1979)
26. Wohlfarth-Bottermann, K.: J. Cell Sci. **16**(1), 23 (1974)
27. Wohlfarth-Bottermann, K.E.: J. Exp. Biol. **81**, 15 (1979)
28. Mayne, R., Adamatzky, A.: Nano LIFE **5**(1), 140007 (2015). doi:10.1142/S179398441450007X
29. Pierron, G.: Proceedings of a NATO Advanced Research Workshop on the Molecular Biology of Physarum Polycephalum. Plenum press (1985), chap. 4: Temporal order of replication and gene expression
30. Adamatzky, A.: Physarum Machines: Making Computers from Slime Mould. World Scientific, Singapore (2010)
31. Fingerle, J., Gradmann, D.: Electrical properties of the plasma membraneof microplasmodia of Physarum polycephalum. J. Membr. Biol. **68**, 67–77 (1982)

32. Yoshiyama, S., Ishigami, M., Nakamura, A., Kohama, K.: Cell Biol. Int. **34**(1), 35 (2010). doi:10.1042/CBI20090158
33. Guttes, E., Guttes, S.: Exp. Cell Res. **20**(1), 239 (1960). doi:10.1016/0014-4827(60)90248-2
34. Nakagaki, T., Yamada, H., Ueda, T.: Biophys. Chem. **84**(3), 195 (2000). doi:10.1016/S0301-4622(00)00108-3
35. Adamatzky, A.: Mater. Sci. Eng.: C **30**(8), 1211 (2010). doi:10.1016/j.msec.2010.06.020
36. Mayne, R., Patton, D., Costello, B., Adamatzky, A., Patton, R.: Int. J. Nanotechnol. Mol. Comput. **3**(3), 1 (2011). doi:10.4018/ijnmc.2011070101. http://services.igi-global.com/resolvedoi/resolve.aspx?doi=10.4018/ijnmc.2011070101
37. Anderson, O.: J. Eukaryot. Microbiol. **40**(1), 67 (1993)
38. Githens, S., Karnovsky, M.L.: J. Cell Biol. **58**(15), 536 (1973)
39. Adamatzky, A.: On attraction of slime mould Physarum polycephalum to plants with sedative properties. Nat. Proc. **10** (2011). doi:10.1038/npre.2011.5985.1
40. Adamatzky, A., Costello, B.D.L.: Physarum attraction: why slime mold behaves as cats do? Communicative Integr. Biol. **5**(3), 297–299 (2012)
41. de Lacy Costello, B.P.J., Adamatzky, A.I.: Communicative Integr. Biol. **6**(5), e25030 (2013). doi:10.4161/cib.25030
42. Jones, J.: From Pattern Formation to Material Computation. Emergence, Complexity and Comutation, Springer (2015)
43. Ueda, T., Hirose, T., Kobatake, Y.: Biophys. J. **11**, 461 (1980)
44. T. Ueda, Y. Kobatake.: The Biology of Physarum and Didymium. Academic Press (1982), Cell Biology: A series of monographs, vol. 1, chap. 4: Chemotaxis in plasmodia of Physarum polycephalum
45. Sauer, H.: Developmental Biology of Physarum. Developmental and Cell Biology Series. Cambridge University Press (1982)
46. Hato, M., Ueda, T., Kurihara, K., Kobatake, Y.: Cell Struct. Funct. **1**, 269 (1976)
47. Mayne, R., Adamatzky, A.: Int. J. Gen. Syst. **44**(3), 305 (2015). doi:10.1080/03081079.2014.997528
48. Wormington, W., Weaver, R.: Proc. Natl. Acad. Sci. USA **73**(11), 3896 (1976)
49. Barrantes, I., Glockner, G., Meyer, S., Marwan, W.: BMC Genomics **11**, 115 (2010). doi:10.1186/1471-2164-11-115
50. Adamatzky, A.: Steering plasmodium with light: Dynamical programming of Physarum machine, (2009). arXiv preprint arXiv:0908.0850
51. Adamatzky, A.: arXiv preprint. arXiv:0908.0850 (2009)
52. Adamatzky, A.: Org. Electron.: Phys. Mater. Appl. **14**(12), 3355 (2013). doi:10.1016/j.orgel.2013.10.004
53. Adamatzky, A.: Sens. Actuators B: Chem. **188**(1), 38 (2013)
54. Mayne, R., Tsompanas, M.A., Sirakoulis, G.C., Adamatzky, A.: Biomed. Eng. Lett. **5**(1), 51 (2015). doi:10.1007/s13534-015-0173-3. http://link.springer.com/10.1007/s13534-015-0173-3
55. Adamatzky, A., Schubert, T.: Mater. Today **17**(2), 86 (2014). doi:10.1016/j.mattod.2014.01.018
56. Anderson, J.: J. Gen. Physiol. **35**(1), 1 (1951)
57. Hato, S.: Integral Biomathics. Springer (2012), chap. Galvanotaxis of the plasmodium of Physarum polycephalum, pp. 57–62
58. Tasaki, I., Kamiya, N.: Protoplasma **39**(3), 333 (1950)
59. Adamatzky, A.: Biomed. Eng. Lett. **3**, 232 (2013)
60. Whiting, J.G., de Lacy Costello, B.P., Adamatzky, A.: Biosystems **128**, 48 (2015). doi:10.1016/j.biosystems.2015.01.009
61. Shirakawa, T.: Soft Computing and Intelligent Systems (SCIS) and 13th International Symposium on Advanced Intelligent Systems (ISIS). IEEE Xplore (2012), pp. 296–300. doi:10.1109/SCIS-ISIS.2012.6505318
62. Goodman, E., Greenebaum, B., Marron, M.: Radiat. Res. **78**, 485 (1976)
63. McCullough, C., Dee, J.: J. Gen. Microbiol. **95**(1), 151 (1976)

64. Daniel, J., Rusch, H.: J. Gen. Microbiol. **25**, 47 (1961)
65. Mohburg, J.: The Biology of Physarum and Didymium. Academic Press (1982), Cell Biology: A series of monographs, vol. 2, chap. 8: Preparation of Spherules
66. Aldrich, H.: The Biology of Physarum and Didymium. Academic Press (1982), Cell Biology: A series of monographs, vol. 2, chap. 23: Culture Methods
67. Ishigami, M., Kuroda, K., Hatano, S.: J. Cell Biol. **105**, 321 (1987)
68. Iwamura, T.: Bot. Mag. **62**, 126 (1949)
69. Ueda, T., Gotz Von Olenhusen, K.: Exp. Cell Res. **116**, 55 (1978)
70. Mayne, R., Adamatzky, A.: On the Computing Potential of Intracellular Vesicles. Plos One. **10**(10), e0139617 (2015). doi:10.1371/journal.pone.0139617