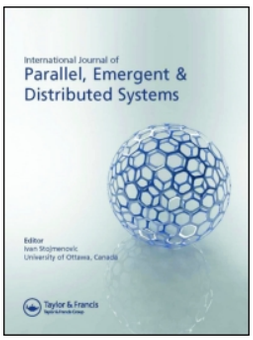

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On coupled oscillator dynamics and incident behaviour patterns in slime mould *Physarum polycephalum*: emergence of wave packets, global streaming clock frequencies and anticipation of periodic stimuli

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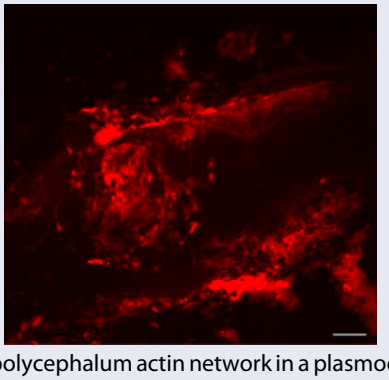
ABSTRACT

Slime mould *Physarum polycephalum* is a single cell which physically oscillates via contraction of actomyosin in order to achieve motility. Several of its apparently 'intelligent' behaviour patterns such as anticipatory responses to periodic stimuli have recently been attributed as functions of the coupling between the oscillating intracellular reactions which drive its rhythmic muscular contraction, but the mechanisms that underlie these phenomena have not yet been experimentally verified. Through laboratory investigations in which we entrain the *P. polycephalum* plasmodium via periodic ultraviolet light exposure we find that this phenomenon is likely to result from biasing its various oscillating life processes through altering local concentration profiles of various allosteric molecules and their effectors. This temporarily overwrites the global streaming clock frequency and eradicates the wave packets usually observed in slime mould biomechanical oscillation. This response is likened to an intracellular chemical memory. We proceed to present a multi-agent model in which we demonstrate that travelling waves and oscillatory clock frequencies may emerge in the virtual organism's biomechanical oscillator, although anticipatory responses cannot be replicated by simple mechanical interactions. We conclude by arguing that these phenomena are best characterised as analogue computation and discuss practical applications therein.



The Physarum polycephalum actin network in a plasmodial tubule. SiR-actin staining, scale bar 200μm.

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KEYWORDS
Physarum polycephalum; anticipation; ultraviolet light; emergent behaviour; multi-agent model; unconventional computing

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1. Introduction

1.1. Epistemological basis of slime mould behaviour classification

Slime mould *Physarum polycephalum*, a single-celled amoeba-like organism formed principally from a network of rhythmically-oscillating caudal protoplasmic tubules when in its vegetative (plasmodial) life cycle stage, was demonstrated in 2008 [46] to reduce its crawling speed in apparent anticipation of a non-existent stimulus following periodic exposure to unfavourable conditions (a combined temperature and humidity alteration). In context with the authors' endeavours to develop *P. polycephalum* into a polymorphic, polyfunctional unconventional computing substrate,[1] this article concerns an investigation into the biomolecular processes that underlie this phenomenon and a consequent characterisation of this behaviour towards the aforementioned objective.

To paraphrase Dubois [12], anticipation is the process by which a system will alter its momentary state based on a prediction which takes into account both past, present and likely future events. In plainer terms, anticipation is not merely an immediate reaction to events that have and are currently occurring, but also requires the system to make reference to a predictive model of the future based on experiential – i.e. memorised – data. Dubois draws a significant distinction between systems wherein all of the data pertaining to this predictive model are situated externally to the system under observation ('weak anticipation') and those which contain internal mechanisms for storing and processing this simulation data ('strong' anticipation); the latter of these concepts may be equated with the term 'autonomy'.

Which type of anticipation could slime mould be said to exhibit? The issue is contentious as, although we are examining a living system, attributing anticipation to a brainless organism risks attracting derision due to casual links to concepts such as intelligence and learning, whose definitions are notoriously subjective. In such an instance a strong epistemological foundation is necessary, for which we refer to Collier's [7] work on anticipatory behaviour in living systems which states, briefly, that autonomy in living systems is equatable to adherence to its apparent[2] teleology, i.e. an organism will act in accordance with its genetically-encoded instructions to act in self-preservation. Crucially, strong anticipation is an emergent feature of biological systems whose elucidation and experimental manipulation are desirable objectives in the design of unconventional computing devices which capitalise on an organism's ability to process environmental data, learn and adapt.

In the absence of a brain or otherwise well-characterised system for coordinating biological processes, how does slime mould construct an internal simulation in order to facilitate strong anticipation? By extension, how may this be simulated and experimentally manipulated?

We here present a literature review on the bases of anticipatory behaviour in slime mould followed by laboratory experimental studies which demonstrate the entrainment of an apparently anticipatory response of the *P. polycephalum* plasmodium following periodic stimulation with ultraviolet (UV) light. We proceed to extrapolate the biomolecular basis of anticipatory behaviour and present simple, flux-based, bottom-up models of oscillatory dynamics before discussing the answers to the aforementioned research questions.

1.2. Anticipation as a function of nonlinear dynamics of coupled oscillators

Many have advanced the hypothesis that anticipatory behaviour arises from the behaviour of coupled oscillators whose degree of synchronisation has been altered in response to repeated, periodic events which perturb the rhythm of at least one constituent oscillator. This viewpoint has been substantiated by myriad demonstrations of coupled oscillator synchronisation, apparent anticipation and lag in simple artificial systems such as coupled Rössler oscillators (in both simulations [5] and in physical circuits [42]), fractal-generating cellular automata [11] and wave phase transitions in the oscillating Belousov–Zhabotinsky reaction [37]. Light-instigated alterations in excitatory and inhibitory nerve relay oscillation which exert control over circadian rhythm-induced activity in *Drosophila* flies [53]

and interactions of light, temperature, blood pressure, gastroenterological signalling and feedback from various brain regions combining to influence food anticipation in mammals [49] are both well-characterised biological examples of this phenomenon. We approach the following investigation with the aim to evaluate the hypothesis that slime mould anticipation is also a function of coupled oscillators which, crucially, suggests that such behaviour is an autonomous process that is not exclusive to multicellular life.

Indeed, in the seminal article on slime mould anticipatory behaviour [46], the authors advanced a partial, non-specific dynamical system model based on oscillator synchronisation, which was later built on by Pershin et al. [41], who suggested that the dynamical history of the organism's oscillators ('stored' as a pressure differential exerted by the organism's cortex; see following section) is a by-product of pressure gradients exerted via actomyosin contraction and environmental conditions. They proceeded to equate the predicted alteration in flow resistance with the phenomenon of variable electrical resistance in memristors and advanced a simulation of slime mould anticipatory behaviour with a circuit containing one resistor, inductor, capacitor and memristor.

Whilst such simulations are doubtless valuable – especially considering that the organism's current-voltage profile is consistent with that of a memristor [14] – this abstraction is unhelpful when attempting to unravel the influencing factors underlying this behaviour. The memristive model also poses certain open questions, such as the issue of whether any endogenous factors maintain the state of the pressure-based 'memory' system, how it persists given the rhythmic anteroposterior contraction of the organism and if the memory response can occur at a constant environmental temperature and pressure.

1.3. Biological preliminaries of *Physarum* oscillators: a review

Despite the aforementioned bases of extant theories of slime mould oscillator behaviour, it has not yet been considered in any great detail which biological processes combine to coordinate these behaviours; we present the following section as a spiritual successor to the seminal historical review of the topic of slime mould oscillators by Wohlfarth-Bottermann [63].

When viewed with a light microscope, plasmodial (protoplasmic) tubules may be observed to oscillate in diameter rhythmically: the organism's endoplasm (hydrodynamic core) rapidly flows anteroposteriorly, changing direction whenever the tubule diameter peaks or troughs [33]. This process, shuttle streaming, serves to distribute the contents of the cytoplasm throughout the organism and contributes to the generation of motive force; net directional movement is achieved via anterior solation and posterior gelation. Tubule contraction is driven by ectoplasmic (vacuolated gel-like cortical region) actomyosin filaments oriented radially, longitudinally and spirally about their circumference [4]. It has been hypothesised, but not yet demonstrated, that actin assembly and disassembly oscillates in synchrony with contraction [3], which is somewhat supported by observations of oscillation in actin-binding proteins such as profilin [39].

When measured at the membrane, plasmodial bioelectrical activity oscillates sinusoidally in synchrony with muscular contractions, although some have noted that they may not always be exactly in phase [4,24]. This does not suggest that the processes are dependent on each other, however. It is unlikely, for example, that any known bioelectrical phenomenon is the trigger of actomyosin contraction [62] and indeed, whilst electrical oscillations were historically thought to be driven by fluctuations in ion distribution as a result of contraction [3,28], they are still present in plasmodia whose contractile activity has been chemically restrained [62].

So what are the key determinants of *P. polycephalum's* membrane potential? Oscillations in the concentration of free intracellular calcium ions ($[Ca^{2+}]_i$) have been repeatedly linked to maintenance of membrane potential and actomyosin contraction [36,47,50] due to their similar periods (and possibly also the well-characterised role of calcium in facilitating excitation–contraction coupling in mammalian muscle cells). It would seem that this is a misconception, however, possibly as a consequence of the contradictory nature of published evidence pertaining to the role of calcium in oscillating plasmodia,

which dates back to a paper by Ridgway and Durham [44], who advanced the hypothesis that $[Ca^{2+}]_i$ oscillation corresponds to both tension generation and electrical oscillation and is hence the cause of both. Their findings were repeatedly called into question [3,63], however, for being based on fluorimetric readings that were not calibrated to compensate for variation in plasmodial thickness (a situation previously cautioned in Ref. [45]). $[Ca^{2+}]_i$ does oscillate with a similar period to actomyosin contraction but in approximate anti-phase [66,67], suggesting conversely that $[Ca^{2+}]_i$ is not a direct determinant of membrane potential and may be involved in the actomyosin relaxation cycle rather than its contraction. In Ref. [67] (whose studies utilised calibrated (ratiometric) fluorimetry), the authors demonstrated that plasmodial $[Ca^{2+}]_i$ oscillates in apparently self-precipitating waves, confirming the earlier hypotheses of Kessler [4]. Calcium waves travel at approximately 5 μm/s, suggesting that they are not propagated by shuttle streaming, which propels the endoplasm at velocities exceeding 1 mm/s [23]. Note also that the majority of the organism's calcium is stored in cytoplasmic vesicles, whose function is similar to the sarcoplasmic reticulum in certain contractile mammalian cells [4,34], the time-varying quantity of which has not been determined with any clarity.

Evidence also exists to suggest that calcium influx may also be pro-contractile, such as the discovery of plasmodial calmodulin, a protein which up-regulates actomyosin contraction at certain (comparatively low) $[Ca^{2+}]_i$ concentrations, although more recent studies have supported calcium-mediated promotion of relaxation via interactions with myosin II and fragmin [25,27,29,30,66,67]. A mathematical model of the plasmodial calcium oscillator by Smith and Saldana [51] assumes the pro-relaxation effects of calcium on actomyosin and though incomplete, their simulations accurately replicated the fluctuations in $[Ca^{2+}]_i$, tension generation and ATP concentration observed in laboratory studies.

Crucially, a great many studies have demonstrated – through the use of chelating agents [32], replacement of the endoplasm with artificial media [59] and limiting the environmental supply of nutrients [64] – that streaming cannot occur in the absence of calcium; coupled with the knowledge of their apparent synchrony, this would seem to imply that the oscillating bioelectrical and biomechanical systems are driven by the same 'clock' mechanism (which is unidentified but historically sought-after [10]) but are otherwise independent of each other. It is assumed that an underlying clock frequency – such as a mean value of the combined frequencies of all tubes within a network – is maintained by the organism and exerts control over tubes which become desynchronised.

Although we acknowledge the apparent concentration-dependent actions of cytoplasmic calcium, since the most widely accepted explanation for the generation and propagation of shuttle streaming is based on purely excitatory effects of calcium (Kessler's 'Stretch Entrainment' hypothesis [4], wherein vessel stretch resulting from dilation during relaxation phases precipitates calcium influx and hence contraction), characterisation of any behaviour patterns on this incompletely-described phenomenon is unwise.

To return to the issue of determinants of bioelectrical potential, trans-plasma membrane transport of sodium, potassium, magnesium, phosphorous and sulphur have also been demonstrated to not be primary determinants [19], but they may modulate the frequency of shuttle streaming. Chemical sensing of nutrient/repellent gradients via membrane-bound receptor binding or electrostatic interactions with the membrane may also influence streaming frequency as well as electrical patterns [17], but for clarification, all previous and consequent elaboration on the subject concerns unstimulated plasmodia. Fingerle and Gradmann [13] advanced strong evidence that the major determinant of membrane potential is in fact the active transport of hydrogen ions across the plasma membrane. H^+ are a by-product of oxidative phosphorylation (i.e. the production of chemical energy in the form of ATP through the breakdown of internalised nutrients). This adequately explains the continuation of electrical oscillation in the absence of streaming, as energy supply mechanisms must necessarily continue unabated at all times. Indeed, plasmodial ATP concentration oscillates in phase with vessel tension production [68] and promotes calcium sequestering within vesicles [4], thereby supporting observations of the phase–anti-phase relationship between ATP/tension production and $[Ca^{2+}]_i$

levels. Both bioelectrical and biomechanical oscillators may therefore be considered as reflections of underlying energy production mechanisms.

There are certain low-frequency (hours–days) periodic phenomena such as the myriad processes that underlie the cell cycle and indeed it is likely that factors such as nuclear division and varying enzyme concentrations will modulate shuttle streaming oscillators to some degree. That said, although factors such as the organism's cell cycle stage may well alter the magnitude of response to a specific stimulus – it is not impossible that regular calcium ion fluctuations over a course of several hours could precipitate gene expression or silencing – it seems unlikely that an open-loop system such as this could coordinate an anticipatory response that rapidly vanishes if periodic stimulation is ceased.

Certain components of the plasmodial signal transduction system are also known to oscillate – most notably, cyclic AMP, which oscillates some 60° preceding the phase of calcium oscillations, although it seems likely that this is a by-product of actomyosin contraction – whereas others, such as adenine nucleotides do not [60]. Instead, the concentrations of these compounds are influenced by direct stimulation of receptors for chemicals, light etc., and influence the excitability of individual oscillating systems.

To summarise this section, the *P. polycephalum* plasmodium contains several well-defined, partially-independent oscillating systems which can be loosely grouped as biomechanical, bioelectrical, metabolic/energy supply system and genetic. The phases of various component oscillators are approximated in Figure 1. Crucially, however, some intracellular compounds which influence oscillatory rhythms do not themselves oscillate (at least in resting conditions). This seems to suggest that this latter category exhibit a form of static biochemical information storage. We hypothesise that if an anticipatory agent is required to construct an image of reality in order to predict future events, repeated stimulation-induced alterations in various biochemical factors that (amongst other roles) alter the characteristics of the coupled oscillators in order to affect proportional responses to said stimuli present a viable mechanism for mediating short-term chemical 'memory'. This is perhaps unsurprising, as *P. polycephalum* has previously been demonstrated to secrete compounds which are used for a function likened to an extracellular memory [43].

2. Experimental methods

2.1. *P. polycephalum* cultivation

Plasmodia of slime mould *P. polycephalum* (strain HU554 × HU560) were cultivated on 2% non-nutrient agar (NNA) in the absence of light at room temperature. Porridge oats were supplied as a nutrient substrate and sub-culturing was performed every 2–4 days, as required.

2.2. Actin visualisation

Samples of plasmodial homogenate were inoculated onto a 0.5 ml NNA hemisphere overlying a large glass microscope coverslip. A second hemisphere was situated 10 mm away on the coverslip which had an oat flake placed on it to act as a chemoattractant. The cover slip was then placed in a sealed Petri dish and left for 24–48 h to allow the organism to propagate between the two hemispheres. This typically resulted in a single plasmodial tubule being formed between the two.

Actin was visualised in these plasmodia in order to correlate the effects of light on actomyosin contraction; a calcium channel blocker, verapamil, was used in some experiments in attempts to decrease fluorescent marker efflux. Actin staining was achieved with SiR-Actin (Spirochrome, Switzerland), which was prepared at a concentration of 100 nM in distilled water and injected into a plasmodial tubule spanning the NNA hemispheres using a glass needle with a 30 μm tip diameter and a CellTram microinjection system (Eppendorf, Germany). Approximately 1 μl of staining solution was delivered. During experiments in which plasma membrane calcium channels were blocked, the injected dye solution also contained 10 μM verapamil. The coverslip was then transferred to a Perkin Elmer Ultraview ERS FRET-H spinning disk confocal microscope for visualisation. Light microscopic

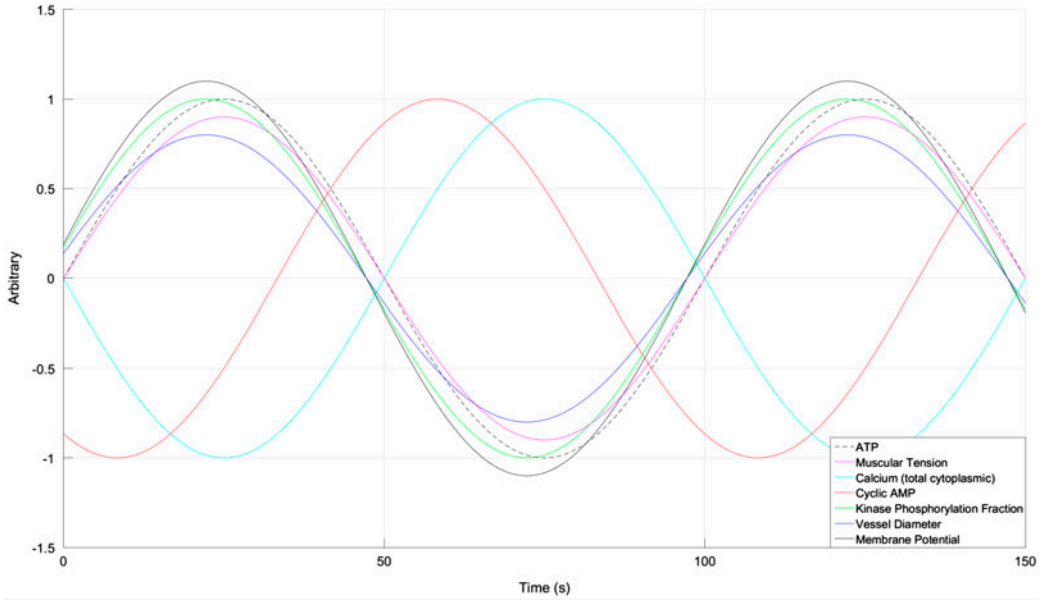

Figure 1. Graph to show phase differences between various plasmodial oscillators. Membrane potential, vessel diameter and kinase phosphorylation fraction are in phase, as are ATP concentration and muscular tension; the latter set follows the former by approximately 10°. Intracellular calcium levels oscillate 180° out-of-phase with ATP/muscular tension and cAMP levels lag approximately 60° behind. Data adapted from Refs. [3,51,63,67].

imaging and analysis was performed with an Olympus SC50 digital camera (Olympus, USA). Confocal microscopic data was processed with Volocity (Improvision, USA) in which images received colour assignment and contrast enhancement. Deconvolution was not used. Unprocessed image files will be made available on request.

2.3. Entrainment experiments

The following experiments were conceived on the principles that *P. polycephalum* exhibits negative phototaxis – i.e. a biophysical response to stimulation enacted by a system of coupled oscillators that can be measured optically – and that it can be entrained into exhibiting apparently anticipatory responses as a result of periodic deleterious stimulation. Plasmodia were cultivated on a 0.5 mm layer of NNA in plastic Petri dishes. Complex, robust tubule network formation was encouraged by allowing the organism to fully colonise its plate, which was scattered with oat flakes. Dishes were then transferred to an inverted light microscope: tubules of approximately 100 μm diameter were selected and visualised under a constant 125 Lux (approx. 1.8×10^3 erg/mm^2) supplied by a 100 W halogen light source. All experiments took place under an opaque cover to prevent interference by external light sources. The area under observation, which was always the same size by virtue of all samples being viewed with the same objective lens (×10), was allowed to equilibrate to the new environment for approximately 15 min before being subjected to periodic stimulation with the microscope's 100 W mercury arc lamp via a 340 nm (UV-A) filter. Scoping experiments demonstrated that using a 165 lux UV source (approx. 2.4×10^3 erg/mm^2) was sufficient to elicit measurable reactions – an increase in streaming frequency and general downward trend in vessel diameter over time – without causing rapid deleterious effects to the organism.

Periodic stimulation of the organism was achieved as follows, where 'stimulation' phases (S_n) were periods of UV light exposure, 'rest' phases (R_n) were periods where no stimulation was given and 'anticipation' phases (A) were of the same duration and periodicity as S phases, but no stimulation was provided:

$$R_1 \rightarrow S_1 \rightarrow R_2 \rightarrow S_2 \rightarrow R_3 \rightarrow S_3 \rightarrow R_4 \rightarrow A$$

Table 1. Table to show dominant frequencies corresponding to dataset in Figure 4. All values in Hz except for ΔAR_4, which shows the percentage increase in streaming frequency between A and R_4 phases. BLF: baseline frequency.

R_1 (BLF)	S_1	R_2	S_2	R_3	S_3	R_4	A	ΔAR_4 (%)
0.013	0.012	0.012	0.009	0.013	0.013	0.012	0.017	141.67

One hundred and twenty seconds was chosen as the duration for the stimulation phase in all experiments as exposure times longer than this tended to result in spontaneous withdrawal of the plasmodial tube under investigation. 900 s was chosen as the length of R periods. The state of the organism's biophysical oscillator was assessed through light microscopic measurement of tubule diameter, which was performed using CellSens software (Olympus, USA) by making periodic (approx. every 14.5 s) measurements of vessel width originating from the same XY coordinate perpendicularly to the tubule margins. Experiments were performed in triplicate. Data were then analysed using Matlab 2015a (Mathworks, USA) fast Fourier transform and periodogram functions.

The effects of treatments were assessed by splitting power spectra of the resulting datasets into separate phases corresponding to each $R/S/A$ phase, which were collated and compared with control results. Furthermore, the recorded change in frequency during each phase was compared with the initial 'baseline' (i.e. clock) streaming frequency, as was measured in the R_1 phase of the experiment; final results were normalised against drift from the baseline frequency in order to isolate the effects of streaming clock alteration, which may or may not have resulted from UV stimulation. Anticipatory responses were therefore indicated by an increase in streaming frequency between R_4 and A phases minus the percentage frequency changes (if any) from mean control measurements for the same phases and baseline drift within the same dataset.

3. Experimental results

3.1. Fluorescence imaging

In corroboration with previous observations [35,61], actin was found to form dense cortical networks about the periphery of plasmodial tubules. Irradiation with an intense orange light source (class IV Ar/Kr laser, 568 nm), consequent of exciting the fluorescent actin probe, was found to cause rapid actomyosin contraction (Figure 2). Sample heating was assumed to be minimal as the environment was temperature controlled and laser exposure was intermittent over a period that typically lasted $\leq$ 4 s. Addition of verapamil was found to result in cessation of streaming and changes in tubule diameter in response to light irradiation, but did result in more intense staining.

3.2. Entrainment experiments

Unstimulated plasmodial tubules (Figure 3(a)) demonstrated periodic (ranging from 0.008 to 0.013 Hz) dilation and contraction organised into wave packets of approximately 0.001 Hz. A general slight upward trend in tubule diameter was observed in most control plasmodia. Corresponding power spectra revealed a range of ordered low frequency peaks, the dominant of which tended to be slower than shuttle streaming frequency but correlated with the wave packets which were observed in all control plasmodia.

Tubules subjected to UV stimulation (Figure 3(b)) displayed a downward trend in tubule diameter in all experiments with a tendency to exhibit progressively chaotic amplitude and frequency patterns. Wave packets were not identified in UV-stimulated plasmodia. Dominant frequency peaks tended towards being lower power than shuttle streaming frequencies.

An exemplar dataset split into R, S and A phase periodograms is shown in Figure 4 and their dominant frequencies are in Table 1. Streaming frequency was found to decrease in initial (first and occasionally second) S phases but by the third S phase would invariably be approximately equal to the

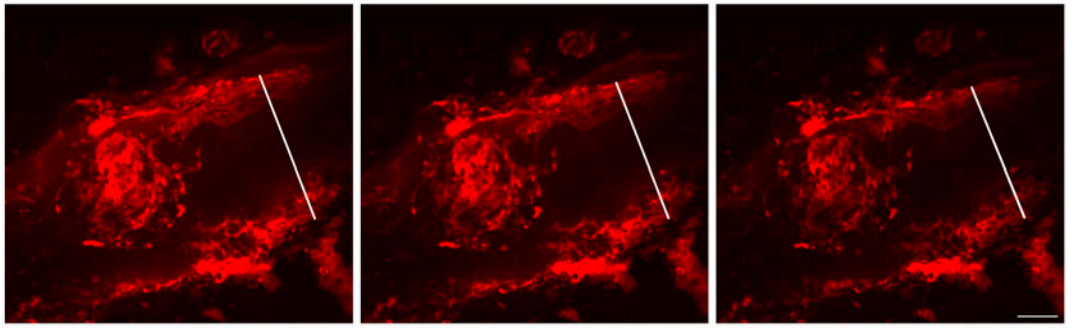

Figure 2. Confocal micrographs demonstrating actin (red) contraction in response to irradiation with a 568 nm laser. Time steps approx 1 s. White bars measure tubule diameter from ectoplasmic margins originating from the same (*x*, *y*) coordinate perpendicular to the vessel's margins (approx. 71°); the vessel shrinks from 1833 to 1731 to 1678 μm (left→right), a decrease of approx. 8%. Alterations in ectoplasmic actin network topology are also evident. Scale bar 250 μm.

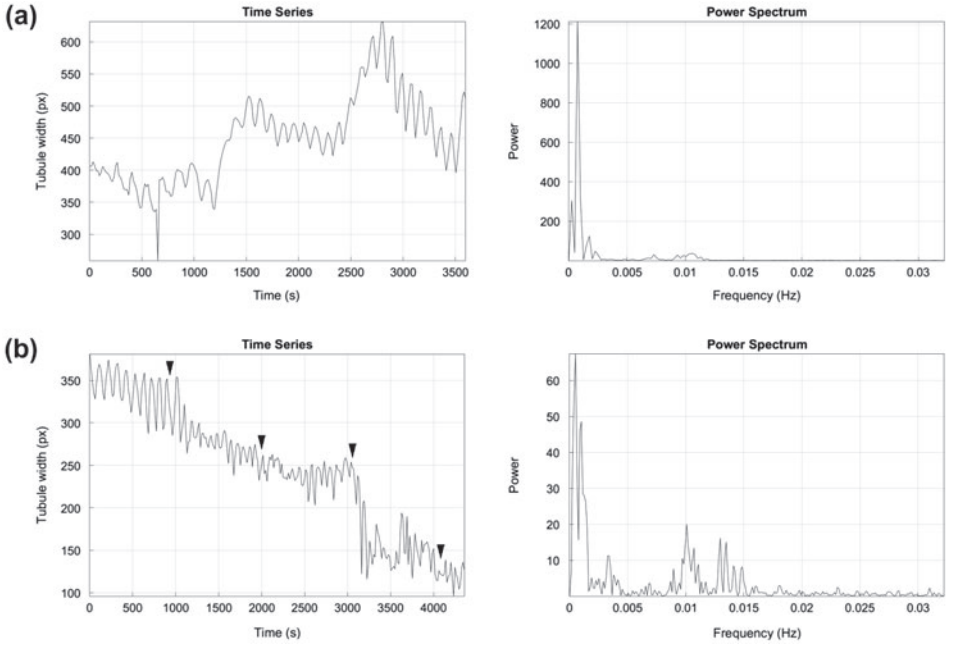

Figure 3. Graphs of exemplar datasets to compare oscillations in tubule diameter and streaming frequencies between (a) Control. Approximately three wave packets are visible. (b) Entrainment experiment. Stimulation and anticipation periods are indicated with arrows (left to right: S1, S2, S4, A). No wave packets are easily discernible.

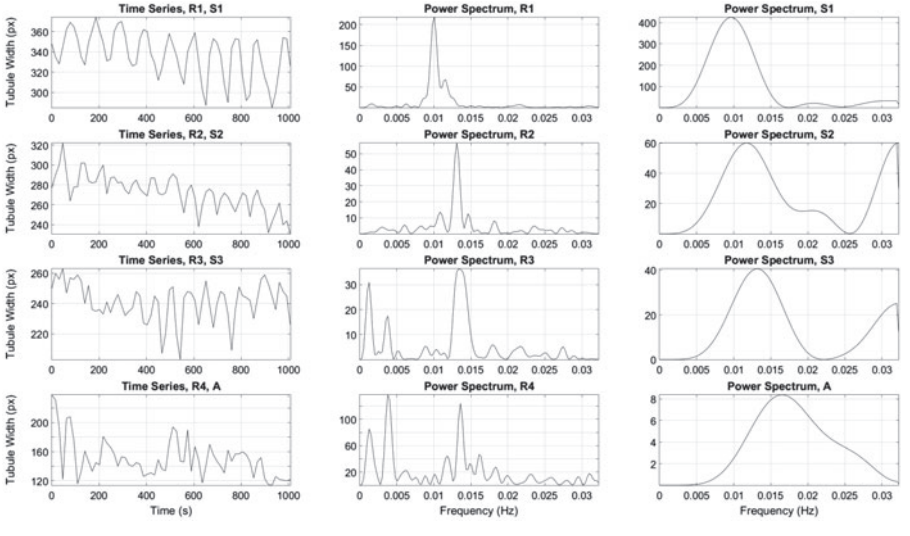

Figure 4. Graphs showing time series and power spectra for each phase of an exemplar dataset. Stimulation (S)/anticipatory (A) periods occur 900 s into their respective time series. Over successive *S* phases, dominant streaming frequencies in both *R* and *S* phases increase but reduce in power in comparison to other emergent frequencies. A frequency increase is evident during the A phase.

dominant streaming frequency in the preceding phase (R_3). *R* phase dominant streaming frequency tended towards remaining constant. Streaming frequency during the *A* phase was found to increase over the dominant frequency of the R_4 phase in all experiments: statistical significance was achieved (Table 2). Mean frequency was found to increase by approximately 27%, both with and without adjusting results to variations in baseline frequency and variation in controls.

Table 2. Collated data to show the mean ($\bar{x}$) and standard deviation (σ) of *A* vs. R_4 phase frequency changes (ΔfA), with and without baseline frequency drift (BFD) adjustment. *P*-values indicate statistical significance of difference in means (unpaired *t*-test, $p < 0.05$).

		ΔfA (%)	$\Delta fA -$ BFD (%)
Control	$\bar{x}$	104.00	6.01
	σ	5.89	8.84
Treatment	$\bar{x}$	131.55	34.23
	σ	14.31	5.98
	p	0.037	0.010
Difference	$\bar{x}$	27.38	27.98
	σ	14.34	5.98

4. Modelling methods: towards a minimal mechanical specification for oscillatory phenomena

As previously mentioned, early analyses of slime mould oscillatory dynamics used interactions between pre-existing mathematical representations of oscillators [46]. Although sufficient to explain some aspects of entrainment, the high-level, 'top-down', description of the oscillators does not take into account the actual *emergence* of the oscillatory phenomena from the low level interactions within the plasmodium. To investigate the emergence of oscillatory behaviour within the plasmodium we employ the particle model first introduced in [20] which has since been used to approximate the behaviour and spatial computation in *Physarum* (see [21] for more information).

The approach uses a population of mobile multi-agent particles with very simple behaviours, residing within a 2D diffusive environment. The discrete lattice stores particle positions and the concentration of a local factor which we refer to generically as chemoattractant. The 'chemoattractant' factor actually represents the hypothetical flux of sol within the plasmodium which is generated by particle movement. Particle positions represent the fixed gel structure (i.e. global pattern) of the plasmodium. The particles act independently and iteration of the particle population is performed randomly to avoid any artifacts from sequential ordering. The behaviour of the particles occurs in two distinct stages, the sensory stage and the motor stage. In the sensory stage, particles sample the local concentration of chemoattractant in the lattice using three forward offset sensors whose angle from the forwards position (the sensor angle parameter, *SA*), and distance from the particle location (sensor offset, *SO*) may be parametrically adjusted. The offset sensors represent the overlapping and intertwining filaments within the plasmodium, generating local coupling of sensory inputs and movement (Figure 5(a)). During the sensory stage each particle changes its orientation to rotate (via the parameter rotation angle, *RA*) towards the strongest local source of chemoattractant (Figure 5(b)).

After the sensory stage, each particle executes the motor stage and attempts to move forwards in its current orientation (an angle from 0 to 360°) by a single pixel forwards. Each lattice site may only store a single particle and particles deposit chemoattractant (5 units, arbitrary value) into the

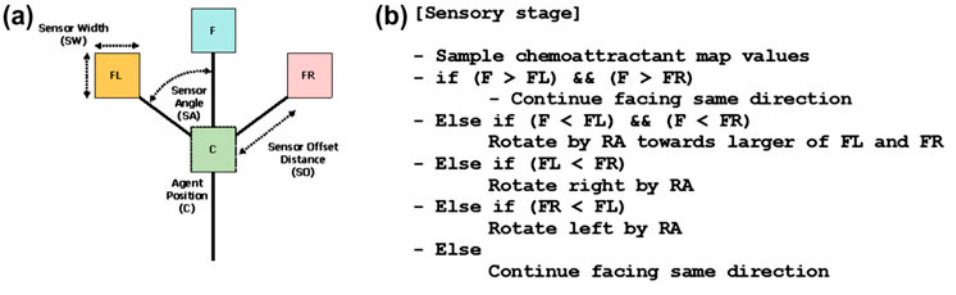

Figure 5. Architecture of a single particle of the virtual material and its sensory algorithm. (a) Morphology showing agent position 'C' and offset sensor positions (FL, F, and FR), (b) Algorithm for particle sensory stage.

lattice only in the event of a successful forwards movement. If the next chosen site is already occupied by another particle the default (i.e. non-oscillatory) behaviour is to abandon the move and select a new random direction. Diffusion of the collective chemoattractant signal is achieved via a simple 3×3 mean filter kernel with a damping parameter (set to 0.01) to ensure strong local coupling of the particles. Movement of particles, and thus movement of the diffusing trail that the particles deposit, corresponds to the flux of sol within the *P. polycephalum* plasmodium. Obstruction of local particle movement corresponds to gelation and resumption of free particle movement corresponds to local solation.

Although the particle model is able to reproduce many of the network-based behaviours seen in the *P. polycephalum* plasmodium such as spontaneous network formation and network minimisation, the default behaviour does not exhibit oscillatory phenomena, as seen in the organism. This is because the default action when a particle is blocked (i.e. when the chosen site is already occupied) is to randomly select a new orientation, resulting in very fluid network evolution, resembling the relaxation evolution of soap films. To reproduce oscillatory dynamics in the particle model requires only a simple change to the motor stage. Instead of randomly selecting a new direction if a move forward is blocked, the particle increments separate internal coordinates until the nearest cell directly in front of the particle becomes vacant, whereupon the particle occupies this vacant cell and deposits chemoattractant into the lattice at the new site. The effect of this change is to remove the fluidity of the default movement of the population. The result is a surging pattern of movement, in which self-organised travelling waves are observed. The strength of the oscillatory dynamics can be damped by a parameter (*pID*, set to 0 for all experiments) which sets the probability of a particle resetting its internal position coordinates, lower values providing stronger inertial movement. When this simple change in motor behaviour is initiated surging movements are seen and oscillatory domains of chemoattractant flux (i.e. transport of trail deposited by the particles) spontaneously appear within the virtual plasmodium showing characteristic behaviours: temporary blockages of particles (gel phase) collapse into sudden localised movement (solation) and vice versa.

The oscillatory domains within the collective themselves undergo complex evolution including competition, phase changes and entrainment. In [58] the model was shown to reproduce the spontaneous formation of oscillatory dynamics observed in small constrained fragments of plasmodia, along with the subsequent transition of oscillatory patterns first reported in [56]. Unconstrained collectives of the model plasmodium were found to generate spontaneous travelling waves capable of generating controllable amoeboid movement [22]. Here we explore the use of the model to study the emergence of oscillatory dynamics and its subsequent competition and entrainment.

5. Modelling results

5.1. Generation and sonification of oscillatory rhythm

Preliminary experiments with the multi-agent model were aimed at generating self-oscillatory phenomena within the particle population, and examining the relationship, competition and entrainment of the oscillatory domains. This was attempted visually and using audio based methods. Sonification of a signal allows the direct experience of phase relationships between separate oscillatory domains and may be considered as a natural mechanism to perceive phase interactions. Sonification of individual regions of the model plasmodium was performed in the following way. We replicated the experiments initially performed by Takamatsu who found that a single strand of *P. polycephalum* plasmodium generated reciprocating oscillations of protoplasmic transport at either end of a tube-patterned environment [55]. We patterned the virtual plasmodium in the same way by inoculating the model as a long strand inside a tube (see Figure 6). The mean amount of particle trail flux within each square region (the squares at each end of the tube in the video recording) was recorded.

We used the Minim Java audio framework (ref: http://code.compartmental.net/) interfaced with the multi-agent model to sonify the flux within the model plasmodium. Each of the values from the

Figure 6. Representation of arena for preliminary model oscillation and sonification experiments. The model population is inoculated and constrained within the black rectangular region. Samples of particle trail flux are recorded from the boxed regions (blue and magenta, online) at each end of the arena.

sampled regions was linked to a separate Minim AudioOutput data structure and patched to separate simple wave generating audio synthesisers whose frequency and amplitude were set to baseline audible preset values using a scaling parameter. Each square region was initially set to identical amplitude (1, the maximum value) and base frequency (100 Hz). The mean flux in each square was added to the base frequency. Because both regions had the same base oscillation frequency and amplitude, any difference in flux in the regions modulated these frequencies and would be perceived as a change in audio phase between the two signals.

Five thousand particles were inoculated within the arena (*SA*90, *RA*22, *SO*15). During initial stages there were random levels of flux within the arena. As particles continued to deposit trails when they moved and sensed their neighbours trail, the flux of particles was locally coupled and regions of different levels of flux emerged. As these regions interacted and become entrained, visible oscillations were seen. Initially, both ends of the tube were in-phase (i.e. had similar flux, at the same time, Figure 7(a)–(c)). After a short time, the competition between oscillations of flux within the arena resulted in a spontaneous shift of oscillation phase and the two ends became anti-phase in visual (and audio, see supplementary recording) oscillations (Figure 7(e) and (f)). A plot of the flux at each end of the arena over time is shown in Figure 7(g), showing the increase in amplitude of oscillations during entrainment and the shift in phase.

5.2. Composition of oscillatory rhythm in the model

Figure 7 clearly shows oscillations in flux between the left and right extremes of the arena. What is the mechanism which generates these global oscillations? Closer analysis of the total flux compared to local particle movement reveals some interesting properties. Figure 8 demonstrates the subtle relationship between particle movement, occupancy and global flux within the arena. Figure 8(a) shows a plot of global oscillation from recordings of the model in a dumbbell shaped arena. The two series in the plot show the flux in the left (blue, online) and right (red, online) chambers of the dumbbell. Figure 8(b), (d), (f), (h) and (j), show the particle occupancy in each side. Red (online) particles are oriented to the right side and yellow (online) particles are oriented to the left side. The timing of these snapshots corresponds to local maxima and minima of the plots of the left chamber in Figure 8(a). Figure 8(c), (e), (g), (i) and (k), show the global trail flux in the arena (brighter corresponds to greater flux) at the same time steps.

The peaks of the plot (and thus flux) for the left chamber correspond to periods of less occupancy (thus greater freedom of movement) in the left chamber. The troughs of the plot for the left chamber correspond to periods of greater occupancy (and thus less freedom of movement). Note that although there is a relatively simple periodic oscillation of global flux values (greyscale images), these oscillations emerge from a more complex flux of particles from one side of the arena to the other. The particles are transported in discrete pulses across the chamber (see supplementary recordings) and the orientation of particles is aligned in patterns which correspond to complex travelling waves connecting the two sides of the arena.

5.3. Modulation of spatio-temporal oscillatory patterns

We continue our previous work described in [58] where oscillation patterns, and transition between different patterns, was observed in *P. polycephalum* and replicated with the model. We now assess

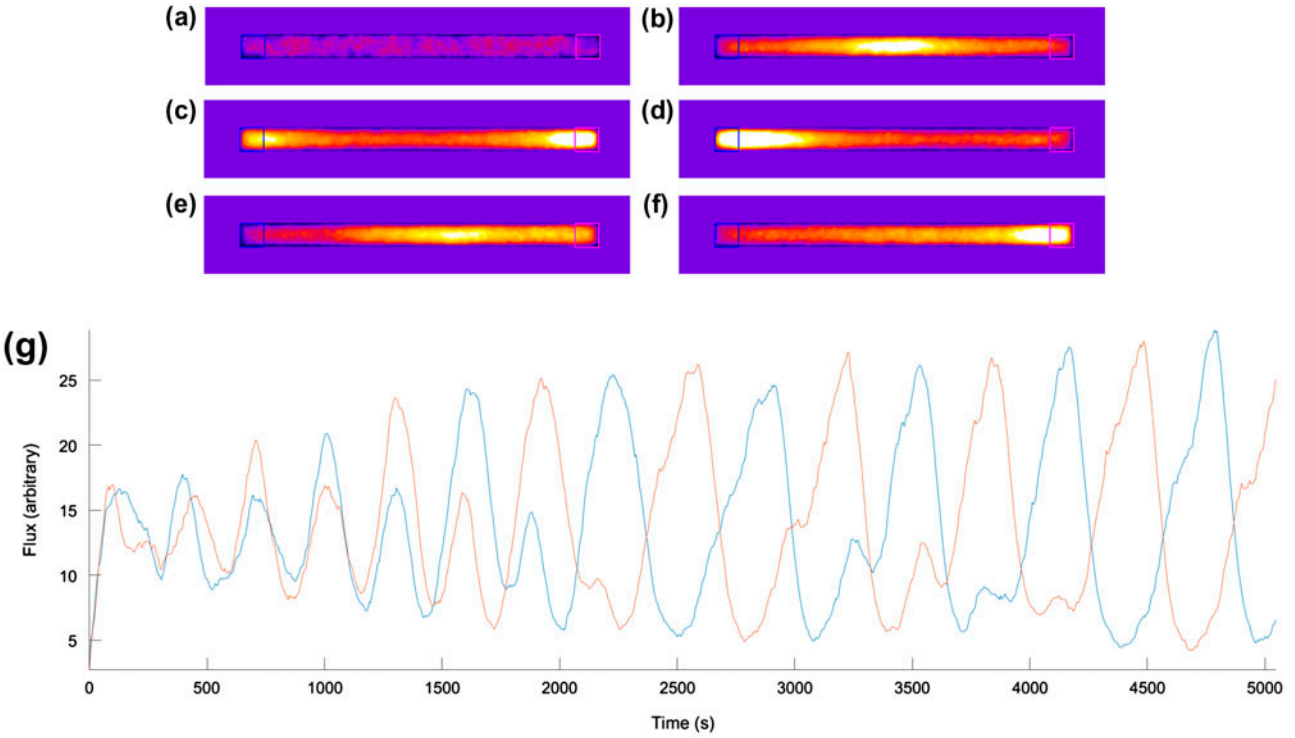

Figure 7. Emergence of oscillatory dynamics and phase interactions in model plasmodium. (a) flux within the plasmodium is initially random and uniform (brighter colours indicate regions of greater flux), (b) and (c) extremal points in the arena initially exhibit in-phase oscillations, (d)–(f) competition and entrainment results in anti-phase oscillations predominating, (g) plot of left (blue) vs. right (red) flux (sampled every five scheduler steps) shows generation of oscillatory rhythm and switch to anti-phase oscillations.

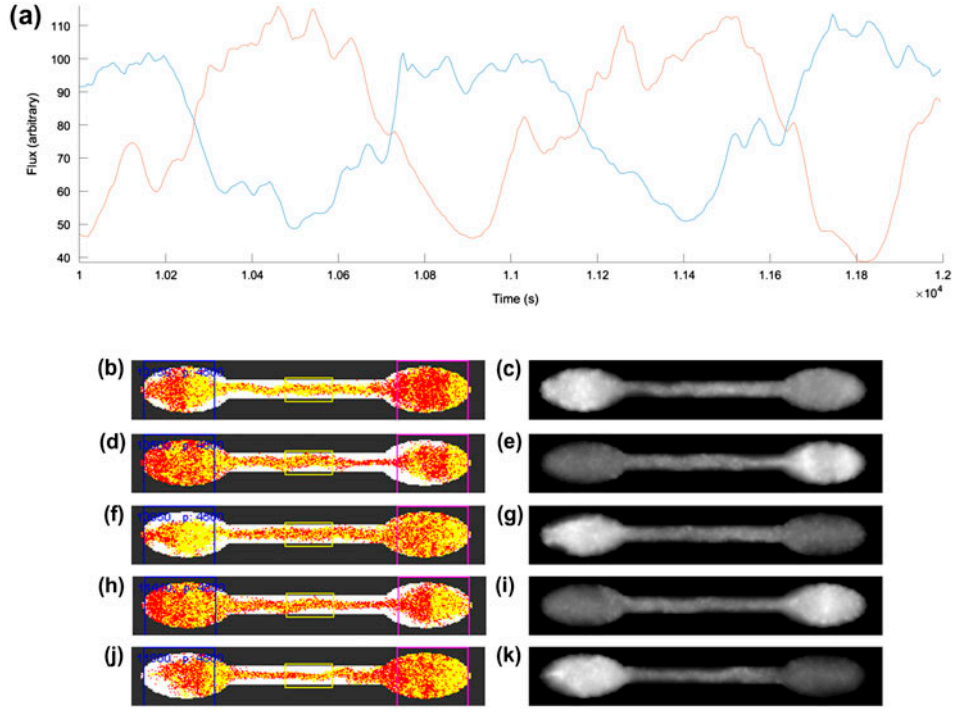

Figure 8. Relationship between global trail flux and local particle movement. (a) plot of flux in left (blue) and right (red) chambers of dumbbell shaped arena, (b), (d), (f), (g), and (j) pattern of particle occupancy and orientation, (c), (e), (g), (i), and (k) pattern of total flux (lighter is greater flux).

whether we can dynamically influence the patterns, specifically their orientation. To assess this behaviour we utilise an annular shaped arena which is inoculated with the virtual plasmodium (Figure 9). Due to the annular shape, any oscillations emergent in one part of the particle population will feed back to all other parts, i.e. there should be rotational oscillation patterns instead of reciprocal oscillation. We then attempt to influence the direction of oscillations by external stimuli.

The response of the plasmodium to external stimuli, in this case laser light stimuli, was demonstrated in Section 3.1. In this section we demonstrate that the spatio-temporal oscillatory patterns in the multi-agent model are also amenable to influence by external stimuli. When inoculated within the annular ring (12,196 particles, *SA*90, *RA*22, *SO*15, Figure 10(a)), the model plasmodium soon formed oscillatory domains (Figure 10(b)) which stabilised into periodic rotating patterns (Figure 10(c) and (d)). The 2D lattice containing the flux values within the multi-agent plasmodium was stimulated with the pattern shown in Figure 10(e) at intervals of 1000, 1050, and 1100 steps with a strong attractant stimulus. This stimulus was sufficient to destroy the coupling which entrained the particles into a stable pattern. After the stimulus was removed, the entrainment began again, resulting in periodic rotation in the opposite direction (Figure 10(f)–(h)). The space-time plots show the time evolution of flux in the circular measuring scheme for both stimulus (Figure 10(i)) and control conditions (Figure 10(j)). The reversal of direction is indicated in the stimulus condition, but not the control condition. It should be noted that the stimulus did always disrupt the entrainment, it did not always result in reversal of the direction of rotation. To guarantee a change in direction may require careful timing of the stimulus (for example, timing the stimulus to match the arrival, or departure, of oscillatory domains). This could be explored in future research.

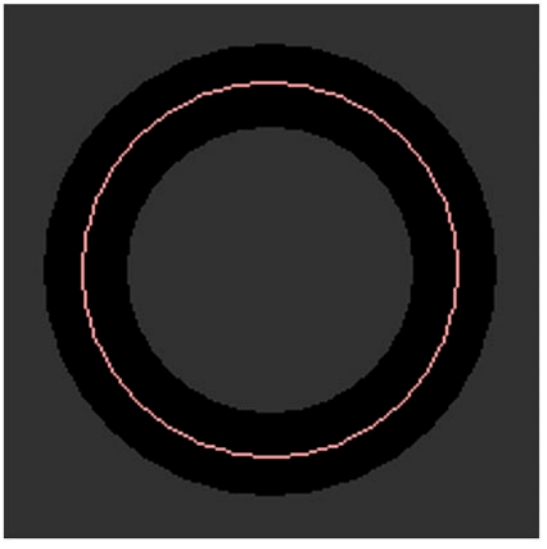

Figure 9. Representation of arena for experiments to study modulation of spatio-temporal oscillatory patterns. Population is inoculated in black region of the annular shape. The circular line at the centre of the ring denotes the locations at which particle trail flux is sampled.

5.4. Inducing frequency changes by geometric constraints

For these experiments we inoculate the particle population in a rectangular arena, as shown in Figure 6. As with the sonification experiments, the end regions of the arena are used to sample particle trail flux. Geometric constraints are generated by introducing a physical blockage into the middle of the area at a pre-determined time, and subsequently removing the obstacle to explore the effect on oscillation frequency.

One method of successfully inducing changes in oscillation frequency was changing the geometry of the arena. An example of this is shown in Figure 11. In this example a single wide horizontal arena is inoculated with the agent population (5000 particles, *SA*90, *RA*22, *SO*15). After an initial period of in-phase oscillations, a strong anti-phase relationship between the left and right measuring points is stabilised (Figure 11(a)). On the introduction of a blockage at the centre of the arena, the model plasmodium is split into two, each acting as a single oscillator (Figure 11(b)). The frequency of these two oscillators is double the frequency of that in the original arena. On removal of the blockage, the particles re-fuse to form the single oscillator with longer period (see plot in Figure 11(c)).

5.5. Inducing frequency changes by transient stimuli

We attempt to modulate the frequency of oscillations in the model using transient stimuli. These stimuli take the form of simulated light irradiation. Any areas of the arena exposed to this stimulus are reduced in flux (by multiplying sensor values with a weighting parameter whose value may be between from 0 to 1). We used a slightly different arena shape, as shown in Figure 12 and as used in [54]. This dumbbell shape was used as it provides a larger region at either side of the arena to 'anchor' the model plasmodium at each end and allow oscillations to propagate whilst maintaining virtual plasmodium distribution throughout the arena. Furthermore, the dumbbell shape was considered to be an analogous conformation to the experimental plasmodia used in the laboratory experimental aspects of this investigation.

Two arena sizes were used. The first was 150×50 pixels (2250 particles) and the second was 300×50 pixels (4500 particles). Strong anti-phase oscillations were observed between the two ends of the arena, characterising the *R* rest periods. Periodic illumination stimuli were introduced into the central

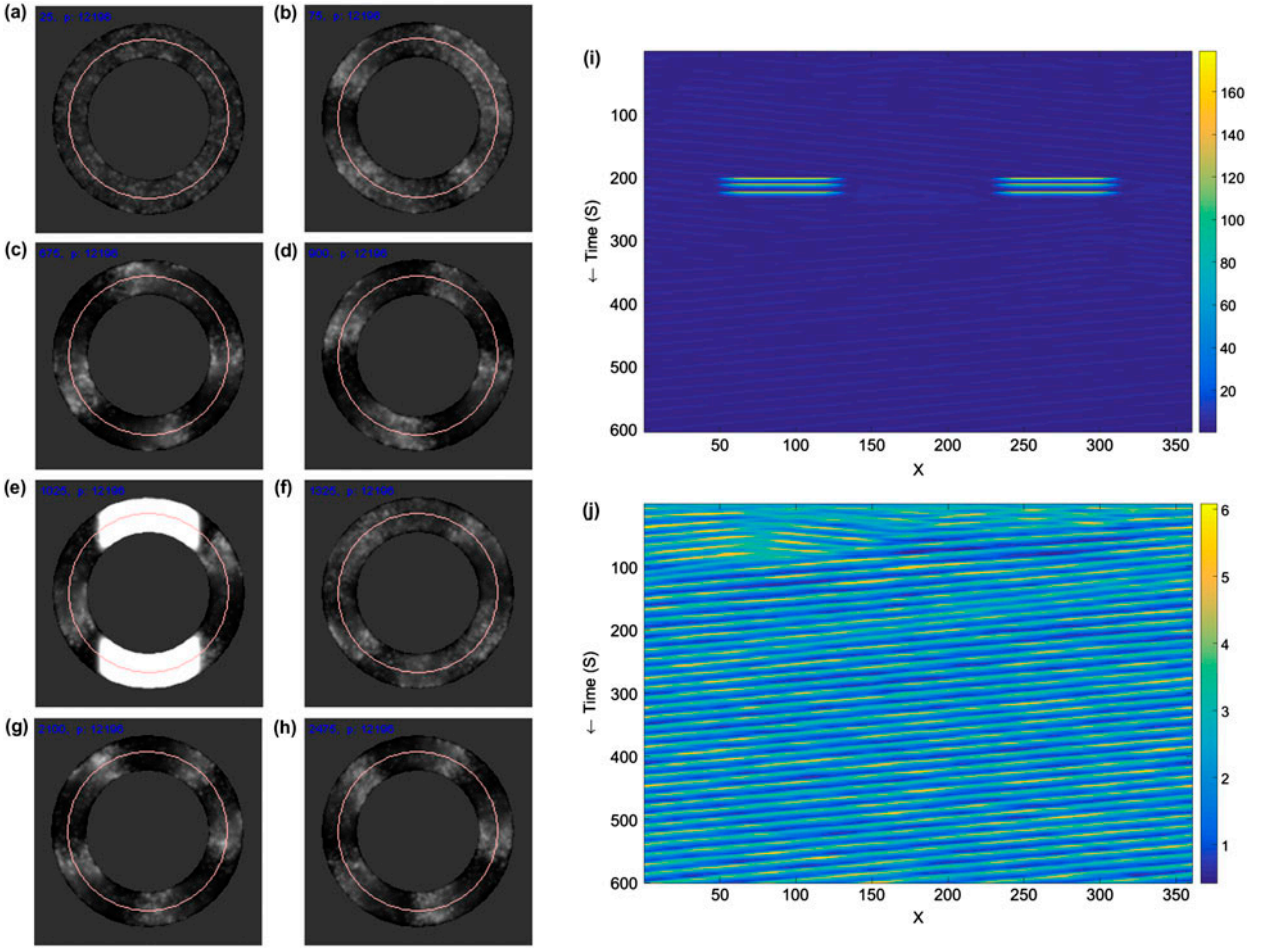

Figure 10. Inducing reversal of oscillation direction with spatio-temporal stimuli. (a) virtual plasmodium is patterned into an annular shape, circular line indicates sampling regions, (b)–(d) oscillatory domains become entrained and a periodic rotating circular motion stabilises, (e) attractant stimuli is presented transiently at 1000, 1050, and 1100 steps, (f) stimulus disrupts oscillation pattern and entrainment begins again, (g) and (h) second entrainment results in a reversal of rotation direction, (i) space-time plot (sampled every five scheduler steps) of circular flux values under stimulus condition, (j) space-time plot of circular flux without stimuli.

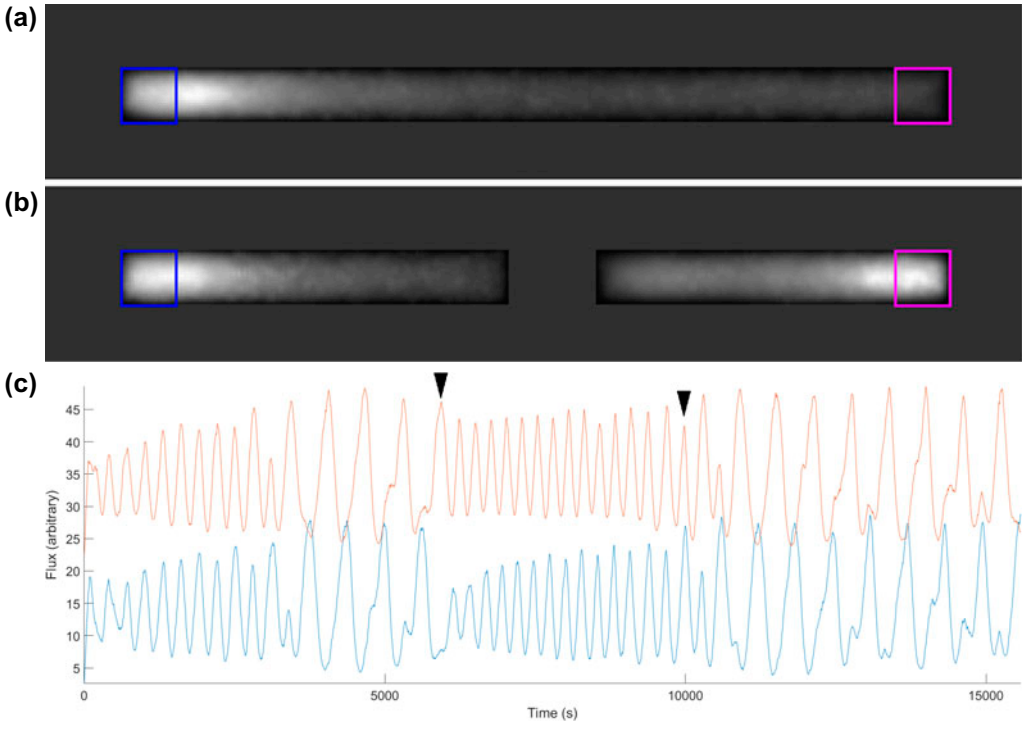

Figure 11. Changing frequency by con-straining arena length. (a) single arena with measuring regions at extreme left and right, (b) introduction of a blockage in the centre of the arena creates two separate oscillators with double the frequency, and (c) plot of flux of left (blue) and right (red) measuring regions (sampled every five scheduler steps) shows increase and decrease in oscillation frequency as the blockage is introduced and removed (arrows indicate timing; left to right: half size, full size). Note that the plots have similar flux values but are offset for clarity.

Figure 12. Representation of arena for experiments to study modulation of frequency by transient stimuli. Population is inoculated in white region of the dumbbell shape. Particle trail flux is sampled from the left (blue, online), middle (yellow, online) and right (magenta, online) regions. Illumination stimuli are introduced in the central (yellow, online) region.

sampling region at 2000, 6000 and 8000 steps for a duration of 2000 steps each time (Figure 13(a)). This corresponded to the stimulus *S* periods of the experimental setup. During the stimulus period, flux at particle sensors in this region was reduced by multiplying sensor values by 0.4. No change in dominant oscillation frequency was observed after the illumination was withdrawn, nor was any frequency difference seen in the 'anticipatory' *A* window in the time series (14000–16000 steps). The control experiments (without any stimuli) showed consistent frequency throughout (Figure 13(b)) which was identical to the stimulus condition *R* periods. The same negative result was seen in both arena sizes and in a number of different *SA* and *RA* sensory parameters, and different *SO* scaling parameter values.

The frequency of the central sampling window was twice that of the left and right windows, but had a noisier profile. The greater frequency in the middle of the dumbbell is due to the transport of particles from one side of the dumbbell to the other.

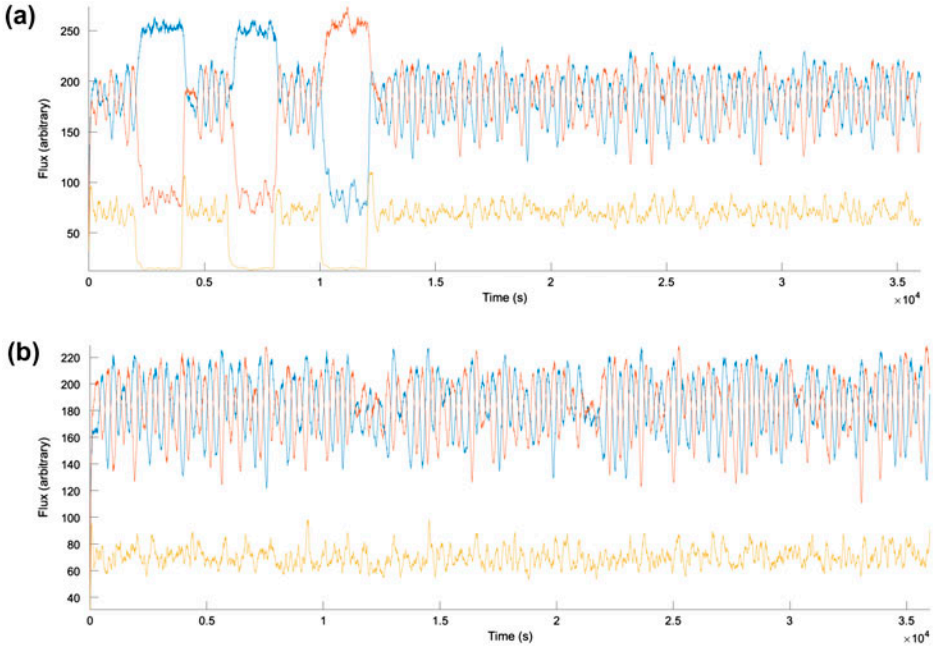

Figure 13. No frequency change or anticipation was observed in response to transient stimuli. (a) plot of flux samples from left (blue, online), right (magenta, online) and middle (yellow, online) regions of the dumbbell arena. Introducing stimuli reduced amplitude of middle region and either one of left and right regions (depending upon which side of the dumbbell had a larger population density) but did not show changed frequency at the 'anticipation' time series and (b) control experiment with no stimuli yielded consistent oscillations and no frequency change.

6. Discussion

6.1. Actin imaging study

Although the light source utilised to excite the fluorescent actin probe was necessarily orange rather than UV, as well as being very intense, the observation of actin contraction in response to light irradiation demonstrates that the organism may rapidly react to photoreception via innervation of its biomechanical oscillator system, thereby indicating the suitability of both light stimulation for directly influencing the various coupled plasmodial oscillators and the use of tubule diameter measurement for assessing the state of the biomechanical oscillator.

The inhibitory effects of the verapamil-induced plasma membrane calcium transport blockade on streaming support historical literature in indicating that preventing the movement of calcium ions across the membrane – and hence, the calcium oscillator – is directly coupled to the biomechanical oscillator, although this of course does not indicate the exact effects of fluctuations in $[Ca^{2+}]_i$.

6.2. Entrainment via periodic UV light exposure

P. polycephalum is known to possess several photoreceptor pigments, at least one of which is receptive to UV light [38,52]; although high doses have been described as initiating sporulation [38,52], the organism's UV photoavoidance suggests that the photoreceptor for these wavelengths initiates a non-specific biochemical pathway which enhances the rate at which the portions of the organism being irradiated migrate away when irradiated with sub-critical doses. This necessitates local actomyosin contraction up-regulation and corresponding interactions with the organism's energy supply system.

Small frequency increases were observed in control plasmodia, possibly as a consequence of white light irradiation and/or illumination-induced heating. The ability to effect a proportional response to optical stimuli implies direct coupling of photoreception and the biomechanical oscillator; this is

perhaps unsurprising, but it does indicate that the mechanisms underlying this behaviour comprise a fairly sophisticated closed-loop control system that is able to detect error in one or more physiological variables and make adjustments accordingly.

Local stimulation of a tubule segment was found to influence the entire tubule's behaviour to such a degree that its streaming rhythm deviated from the initial clock frequency, but further experimentation is required in order to elucidate how the global clock mechanism changes over time and in response to local stimulation. Indeed, historical data indicates that tubules which have been isolated and transplanted onto different plasmodia eventually synchronise (within 30–60 min) with the majority value [65] which seems to indicate that local control mechanisms may override the clock frequency, but only temporarily. This is in agreement with our observation of the disappearance of wave packets from UV-stimulated plasmodia as their underlying cause – superposition of waves of a similar frequency throughout the tubule network – is disrupted when one tubule's frequency is drastically altered.

Statistically significant increases were observed in streaming frequency during *A* phases, which were found to accelerate by approximately 27%, even after adjusting for the slight increases in clock frequency observed in control plasmodia as well as baseline drift. This is indicative of an 'anticipated' response to periodic UV light irradiation.

The nature of the organism's response was surprising as it was contrary to their initial response to UV stimulation during the S_1 and occasionally S_2 phases, which then tended towards becoming approximately equal to the (albeit altered) dominant streaming frequency by the S_3 phase: indeed, during the lag before apparently compensatory measures were put into effect by the organism in the first *S* phases, UV irradiation appeared to suppress streaming frequency. Coupled with the observation that tubules typically reduced in diameter over the course of the experiment, it seems likely that the function of this mechanism is to reduce the organism's exposure to the insulting stimulus via promoting a gradual retreat from the area, the rate of which is presumably proportional to the degree of stimulation.

To address the biomolecular phenomena underlying this behaviour, let us consider what is known about the organism's responses to UV light. UV irradiation may delay plasmodial mitosis and may even cause the mitotic stage to reverse, likely due to DNA damage which is evident in a measurable decrease in the organism's total DNA content [8,9]. As plasmodia undergoing mitosis are known to significantly reduce their streaming speed, it is feasible to propose that periodic UV exposure may arrest the cell cycle to some degree [16] and hence lead to a gradual increase in streaming frequency, but given that *P. polycephalum* mitoses are circa 8 h in duration, this may be discounted as an explanation for the events observed here (compared to approx 1.5 h in our experiments). Furthermore, genetic control systems are open-loop and it seems unlikely that a sufficient proportion of the cell's myriad nuclei would have become altered by the treatments administered in order to effect a global response.

The other known mechanism by which *P. polycephalum* interprets UV irradiation – stimulation of cytoplasmic photoreceptors that initiate signal transduction cascades upon entering a high-energy state – would therefore seem to be the system by which this behaviour emerges. As the classes of biological molecule involved in signalling cascades (allosteric proteins and related effectors) are able to alter the organism's response to an insulting stimulus in such a rapid timescale, we may hypothesise that periodic UV stimulation alters the recruitment/activation of such compounds via second messenger pathways, which in turn influences the behavioural patterns observed. Indeed, the concentration profiles of some such compounds (e.g. protein kinase C) have been observed to oscillate in the *P. polycephalum* plasmodium [3,26]: as such, it would appear that modification of coupled oscillators by directly biasing one or more fundamental component oscillators in the organism's energy supply system is a potential explanation for both the incidence of anticipatory behaviour – i.e. streaming frequency changes in phase with the concentration profile of at least one molecule produced in response to periodic stimulation – as well as the reciprocal modification of other, more easily observable oscillating systems. Note, however, that the concentrations of effector molecules such as those produced in second messenger pathways need not necessarily oscillate in order to influence other cellular systems.

Thus we can address the first research question detailed in Section 1.1: under the tenets of the above hypothesis, the organism is coupled to its environment by a transient, dynamical chemical memory of rapid-reaction biomolecules which alter its behaviour through biasing certain intracellular oscillators which can be entrained to the period of exogenous stimuli. As such, the incident behavioural changes occur whether an adverse stimulus occurs or not. This is consistent with both Dubois' and Collier's definitions of 'strong' anticipation. Our proposal is in partial agreement with previous theories regarding slime mould anticipation but offers evidence towards a previously unconsidered mechanism. The electrochemical repercussions of variation in these 'transient memory' compounds (either directly or via their interactions with energy supply and biomechanical oscillators) appear to be compatible with Gale's [14] observations of memristive behaviour of plasmodial membrane potential.

6.3. Modelling vs. experimental results

The modelling experiments attempted to explore the minimum mechanical requisites for oscillatory phenomena to emerge. The low-level and purely local interactions between individual particles resulted in the emergence of localised oscillatory regimes in the form of travelling waves. Local competition between these waves resulted in entrainment, an increase in amplitude, and ultimately formed global oscillatory rhythms with a stable frequency. Both in-phase and anti-phase rhythms (and transitions between the two) were observed, as noted in *P. polycephalum* itself [55]. Closer investigation of particle movement revealed the more complex relationship between the fast travelling waves emergent in the particle population and the slower long-term changes in reciprocal flux at either end of the arena. These results indicate that a purely mechanical method is sufficient to explain certain aspects of oscillatory dynamics.

What are the properties and limitations of this mechanistic approach to oscillatory dynamics? We found that the direction of spatio-temporal travelling waves could be halted and even reversed by pulsed attractant stimuli. This reproduces the work on controlled modulation of *P. polycephalum* oscillatory rhythm by discrete light pulses reported in Ref. [57]. The stimulus in the model temporarily de-couples local particle flow and the re-establishment of the rhythm by local entrainment is – on occasion – in the reverse direction. To ensure reliable directional changes requires future investigation into the nature and timing of the stimulus pulses.

The oscillation frequency in the model could only be manipulated by *geometric* constraints, i.e. the changing of arena dimensions. When transient stimuli approximating hazardous light irradiation were introduced, there was no change in oscillation frequency, and no corresponding anticipation period was observed. It was suggested in [48] that it is not possible to approximate the individual (vs collective) so-called *atomic* actions of *P. polycephalum*. The modelling results strongly suggest that simple mechanical interactions in response to attractant stimuli may not be sufficient to induce frequency changes in *P. polycephalum*, which must therefore be mediated by cellular changes within the plasmodium, as was suggested by our laboratory experimental results.

Parenthetically, as the behaviour of *P. polycephalum* in our experiments is analogous to certain disease states in oscillating human organ systems, we propose that further work on modelling coupled oscillator behaviour with multi-agent techniques is a viable route towards computer-aided analysis of these conditions. For example, stomach contraction frequency (measured through cutaneous electrogastrogram) is raised (tachygastria) through certain diseases that are associated with up-regulated adrenergic or/and cholinergic neural activity, e.g. irritable bowel syndrome, motion sickness [15,18,31,40]; if the underlying mechanisms of this behaviour are abstracted as coupled oscillators in the same manner as our treatment of *P. polycephalum* (biomechanical, electrochemical etc.), we find that frequency modulation is the result of biasing one component oscillator, i.e. neural innervation, and hence is directly comparable to our model if sources of inhibition were represented geometrically.

6.4. On computing with intracellular oscillators

We propose that the phenomena partially elucidated here are best characterised in the language of computation, although not necessarily in terms of traditional logic. For example, when viewed as a discrete device, the anticipatory response detailed here is a 3-input AND gate wherein the device will output a logical $_{\text{truth}}$ (interpreted as a frequency shift during the *A* period) if and only if all three inputs were provided during *S* phases; such a device would be impractical, although this is nevertheless a demonstration of information encoding (encompassing transduction, structuring, transmission and storage) and by extension, a reflection of intracellular events which may be characterised in the language of computation.

What we have described as *P. polycephalum's* chemical memory is in essence an integrator, as the magnitude of the bias exerted on the 'signal generators' (various oscillating systems) is dictated by the sum of 'calculations' performed by the organism in response to the input it receives. The complete slime mould system, when abstracted to this degree, is an analogue computer. The use of this class of architecture (including derived paradigms, such as neural networks) are currently the subject of scrutiny as interest in unconventional computing substrates rises in anticipation of the looming 'kT', or miniaturisation, barrier faced by silicon-based systems.

The implications of this are twofold: firstly, this lends insights into the workings of other computing-like processes in eukaryotic cells (e.g. mammalian neurons). Secondly, this suggests that biological substrates may be manipulated towards developing viable heteroic artificial-living computing circuitry (although we are of course not the first to suggest this).

For example, if the emergent streaming frequencies imparted to a plasmodium through repeated UV stimulation were found to be both repeatable and directly proportional to stimulus intensity and/or period, then it would be demonstrated that the organism is undertaking arithmetical operations. Blakey [6] hypothesised that it may be possible to entrain plasmodia with periodic functions and derive the period through analysis of membrane potential frequency; if further work in this direction proved fruitful, this could lead to efficient live cell-derived implementations of problem classes that can only be calculated inefficiently with conventional architectures (e.g. factorisation). Further work towards this goal should focus on deducing the limitations of the organism's ability to respond to stimuli for differing periods of time and/or frequency intensities; in scoping studies, for example, it was found that by increasing UV light intensity by 25% and the period of *S* phases to 900 s, anticipatory frequency increases of up to 287.30% (after adjusting for baseline drift; data not shown) were observed, although the rate of tube retreat and result variability were both sufficiently high such that statistically significant results were not achieved.

7. Conclusions

Our imaging results confirm the pro-contractile effect of light irradiation on *P. polycephalum* acto-myosin networks and the necessity of plasmalemma calcium transport for the maintenance of shuttle streaming.

Through our laboratory experiments, we find that the *P. polycephalum* plasmodium may be entrained to increase the frequency of shuttle streaming in anticipation of periodic stimulation by UV light. This temporarily overrides the global 'clock' mechanism in localised areas and in doing so eradicates streaming frequency wave packets. This behaviour is likely to result from biasing one or more coupled biochemical oscillators through eliciting local chemical changes; this can be equated with the concept of 'memory'.

Our model is able to replicate the emergence of a global streaming frequency and wave packets but not anticipatory behaviours, indicating that the latter is not caused by simple mechanical interactions and hence supporting the experimental work presented. Nevertheless, our model is still likely to be applicable to further fields of study which involve oscillating systems.

We propose that slime mould anticipatory behaviours are best characterised in terms of an analogue computer, for which we have demonstrated an example of how this can be used to implement logic and/or memory responses. If developed sufficiently, such a technology could potentially exploit the inherent massive parallelism of biological substrates towards finding efficient implementations of novel algorithm classes that conventional architectures are not well suited to computing.

Notes

1. We refer the reader to Refs. [1,2,34,35] for an introduction to the field of '*Physarum* computing'.
2. Despite the tentative reference to teleonomy with this phrasing, we concede that it is largely irrelevant whether an organism is actually or apparently purposeful when discussing its autonomy as it is nevertheless still 'self-guided' by internal mechanisms.

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Disclosure statement

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