***Physarum polycephalum* as an Active Mechanical Medium**

1. Introduction

One of the current challenges in Biology is to understand how living systems self-organize to achieve emergent properties that improve fitness. In order to act coherently as a unit, each organism must display system-level dynamics that span it. As organisms evolve and become more sophisticated, they must be able to develop increasingly complex organism-wide behaviors that result from the local activities and interactions of subparts. This can lead to a hierarchy of structures that integrate system behavior. At the cellular scale, these can correspond to metabolic networks or cell signaling networks. In higher organisms, it involves the development of integrating systems that can ensure, for example, the distribution of nutrients, the coherent motion of the individual as a whole, or some level of centralized control. In simpler organisms, however, such coordination of subparts required to integrate system activity, referred to here as *coherent dynamics* (CD), must be achieved without requiring complex developmental structures. Furthermore, from an evolutionary perspective, various forms of CD were likely required before organisms had evolved integrating structures. Life must therefore take advantage of biophysical mechanisms that spontaneously lead to CD, either as a final solution to achieve organism-level behavior in simple cases or as a step in developing the required integrating systems. Understanding these mechanisms could not only lead to a detailed description of certain biophysical processes in simple organisms, but it could also provide key insights into the underlying principles that lead to the emergence of structure in living systems, in general.

Biological systems often take advantage of physical phenomena to function. Some examples include: capillary forces used to distribute water and nutrients, gravitational forces that help structure developing organisms, and thermodynamic processes used for thermal regulation. It is therefore natural to expect that CD could take advantage of the various physical mechanisms that are known to produce coherent long-range dynamics and patterns spanning beyond the local interaction scale. Note that, in this context, pattern-formation can also be viewed as the development of extended coherent biological structures. Turing patterns, for example, appear generically in reaction-diffusion systems and produce ordered arrangements that span the system, which have been linked to the morphogenesis of various biological structures. Other physical mechanisms, such as phase separation or convection, have also been related to morphogenesis [1,2]. More recently, it has been shown that systems composed of *active matter* (where mechanical energy is produced locally throughout the system, without central control), and with elastic properties, can develop persistent CD resulting from the interplay between elasticity and activity [3]. Interestingly, different physical mechanisms will produce CD with different characteristics. For instance, the typical correlation scales of CD can result in some cases from the properties of the medium where they develop [4,5] and in others from its boundary conditions [6–8]. This scale may also be either fixed or evolve slowly over time (e.g. through a coarsening process). While the relationship between mechanisms for pattern-formation, CD, and morphogenesis has been studied for several decades, there are still very few model systems and experiments where it can be analyzed in detail [9,10]. Where studies have been made, in most cases the underlying structuring dynamics are only a small part of a complex morphogenetic process where evolution has added several layers of control, such as specifically tailored chemical signaling or metabolic pathways.

The purpose of this proposal is to further our understanding of the relationship between biophysical self-organizing mechanisms, the emergence of CD in living systems, the role of such dynamics in developing biological functions, and their effects on fitness. From an experimental perspective, we aim to achieve this objective by focusing on a relatively simple novel model organism, and employing a laboratory setup that allows unprecedented levels of monitoring and manipulation, in order to develop and test quantitative hypotheses. From a theoretical perspective, we will integrate current qualitative knowledge on the potential role of pattern formation in biological structures and on the emergence of CD in active viscoelastic living systems, into the quantitative analysis, prediction, and control of our experimental system.

The project will focus on the slime mold *Physarum polycephalum* (or 'PP'); a macroscopic, unicellular, blob-like amoeba (shown in Fig 1). This protist oozes through the leaf litter of damp forests worldwide, engulfing and digesting its prey of fungi, bacteria and decaying organic matter. Although the cell may only move at a top speed of a few centimeters per hour, its contents rhythmically stream back and forth at the fastest rate known for any organism, as parts of the cell membrane expand and contract in an oscillatory fashion [11]. Contractions are implemented by myosin and actin filaments, just as in human muscle tissue, and occur at a resting rate of about once every minute [12]. Several mechanisms allow these oscillations to react to environmental stimuli, including internal hydrostatic pressures, and the deformation of the membrane. These mechanisms, together with complex interactions among multiple simple parts, lead to emergent behaviors at the organism level that favor its survival. In biophysical terms, CD arises in PP through a combination of viscoelastic couplings, biochemical signaling, and the local activity of actin filaments that produces localized mechanical energy. Hence, PP is an ideal model organism for the study of the origins and role of CD in biological active matter and living systems, as the local dynamics (viscoelastic flows, mechanical forces, chemical changes) are tightly coupled to emerging structures and sophisticated organism-level function that can be tracked theoretically and experimentally in detail.

Mechanisms for pattern formation and CD in nonequilibrium physical systems have long been considered as the potential origin of various biological structures. While it is suspected that such mechanisms are behind various processes in morphogenesis, there are very few examples where this link has been fully understood. In recent years, there has also been a steady development of the theory of active matter, which encompasses a broad range of systems where the collective dynamics result from individual components that inject mechanical energy at the smallest scales considered. Examples in biology include intracellular actin cytoskeletal assembly and disassembly [3,13–15], developing cell tissues [16–20], fluids with swimming bacteria [21–23], insect swarms [24,25] and bird flocks [26,27]. While the dynamics of active systems can be directly studied in detail (due to their observable spatial and mechanical effects), their potential role in the self-organization of biological systems is only starting to be unveiled. The proposed project will allow us to develop a timely combination of the current knowledge on pattern formation, its hypothesized link to biological structures, the developing theories of active matter, and current efforts to extend them to viscoelastic systems. This work will be structured around our analysis of PP, for which we will develop a novel experimental framework allowing the direct investigation and control of the emergence of patterns and CD. PP is the ideal biological model system for this purpose, with relatively simple architecture and reduced developmental structures, an active viscoelastic origin of its dynamics, a lack of centralized control systems, and a known ability to develop relatively sophisticated organism-wide behaviors (such as decision making [28–32] and pathway optimization [33–35]). These features will allow us to investigate in detail the connection between underlying physical mechanisms that generate CD in nonequilibrium systems and the emergence of structural and functional properties that increase fitness in living organisms.

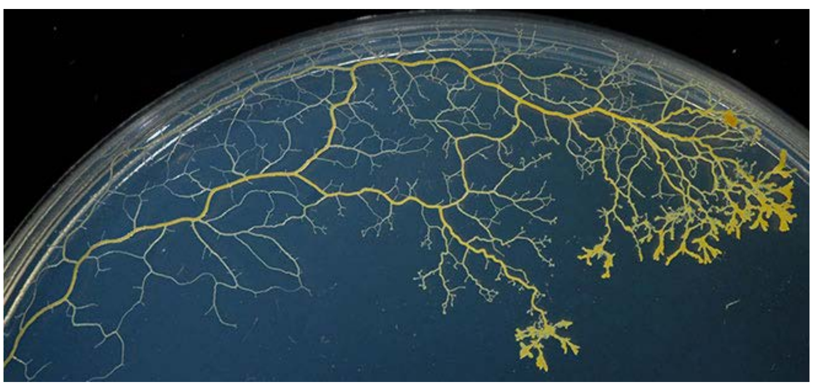

Figure 1. *Physarum polycephalum* cell on an agar petri dish. The cell's emergent coherent dynamics drive the development of an extending network shape. In this case the cell, naturally colored yellow, is exploring from left to right, first building a dendritic network at the search front (right), and then collapsing to just a few transport tubules (left) as the cell moves on (image courtesy Malcolm Ricketts).

2. Project Overview

The proposed project will explore in a simple novel model system, the slime mold *Physarum polycephalum*, how system-wide evolved biological behavior relates to underlying self-organizing physical processes that lead to coherent dynamics.

We will thus link the biophysical mechanisms leading to self-organization to the organism-level behavior of PP by achieving the following three central objectives.

- Build realistic biophysical models of PP dynamics by combining specifically tailored experiments with recent progress in the theory of active matter
- Analyze theoretically and experimentally the various mechanisms for pattern formation and self-organization that could lead to CD in PP
- Link the underlying dynamics of the function and behavior of PP to its known ability to solve survival challenges, including optimization and decision making

Each one of these objectives will be addressed by combining experimental and theoretical approaches through a close collaboration between the three groups involved. The first objective will build upon current active matter theories, known features of the PP biology, and new detailed experimental measurements on its rheology, structure, and behavior to develop mathematical descriptions of the organism's dynamics that can be solved numerically and analytically. The second objective will draw from the large literature on pattern formation and self-organization to identify potential mechanisms that may lead to CD, and test experimentally and theoretically their role in the PP dynamics. The third objective will study how these underlying processes lead to the observed behavior when subject to specific stimuli, environments, and boundary conditions, and how these CD relate to the organism's fitness. A strong overarching motivation is to understand if and how evolution can use the emergent dynamics that result from physical processes to develop complex organism-wide behaviors that benefit survival.

3. Key Approaches

Each one of our objectives above will be addressed through a combination of experiments and theory. Before detailing the specific questions considered, we will describe in this section the key experimental and theoretical approaches that will be used in the project.

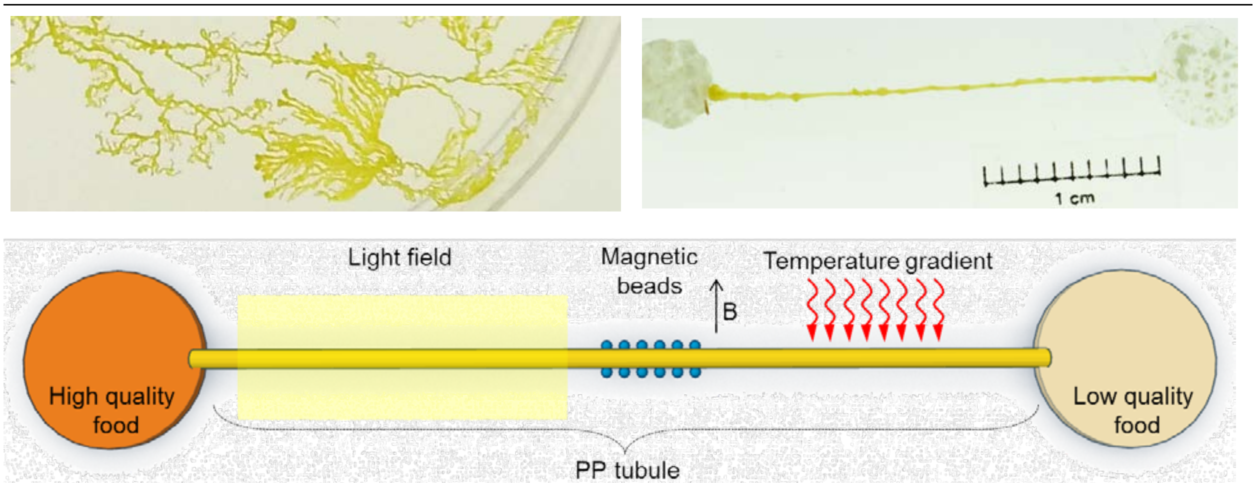

Figure 2. Top left: PP network grown on water surface at PI Garnier's lab. Top right: Isolated PP tubule cut from original network and aligned between two food sources at PI Garnier's lab. Bottom: Schema of proposed perturbations, which include light stimulation, forced deformation of the tubule membrane through magnetic traction of attached magnetic beads, and temperature stimulation.

3.1. Experimental Framework

The slime mold *Physarum polycephalum* is a macroscopic unicellular organism that self-organizes into a network of plasmodial tubules composed of an outer gel that confines and actively pumps the inner sol or cytoplasm through periodic peristaltic contractions [36]. Protoplasmic sol extracts spontaneously reform into plasmodial droplets that exhibit a wide range of spatiotemporal dynamics, including membrane oscillation patterns [37]. These patterns change with the local quality of the environment in areas surrounding the cell [12]. Areas that are attractive, such as those containing food, are sensed by receptors on the cells surface, which induces higher oscillation frequencies locally [31]. In addition, the binding of attractant molecules to sections of the surface membrane reduces the tension at that section, leading to a difference in internal hydrostatic pressure, which in turn produce cytoplasm flows toward the source of the attractants [12]. When repellents such as salts or light are detected, the local oscillation frequency decreases and membrane tension increases, causing cytoplasm to flow away from the repellent zone. Moreover, individual regions of the membrane entrain neighboring regions to pulse at their frequency, providing a mechanism for information propagation throughout the cell [38].

Our project will explore the connection between local active PP dynamics, its resulting coherent dynamical states, and systems biology processes that develop at the organism level, by closely combining experiments carried out at Garnier's Swarm Lab with theoretical analyses by Henkes and Huepe. Our experimental analyses will benefit from our ability to closely monitor the organism's dynamics while fully controlling the stimuli and boundary conditions that define its environment. This level of monitoring and control will play a key role at various stages of our experimental analyses. In the initial stages, it will allow us to measure the passive properties of PP and its stimulus-response dynamics almost as if it were a physical system (while it is in fact a complex biological organism), and thus allow us to develop and test detailed models. In later stages, it will allow us to create automated feedback loops that link instantaneously measured responses to new stimuli, and then use elements of control theory to fully understand the system by developing our ability to manipulate it at unprecedented levels.

We detail below various measurement and manipulation techniques that will be used in our experimental framework.

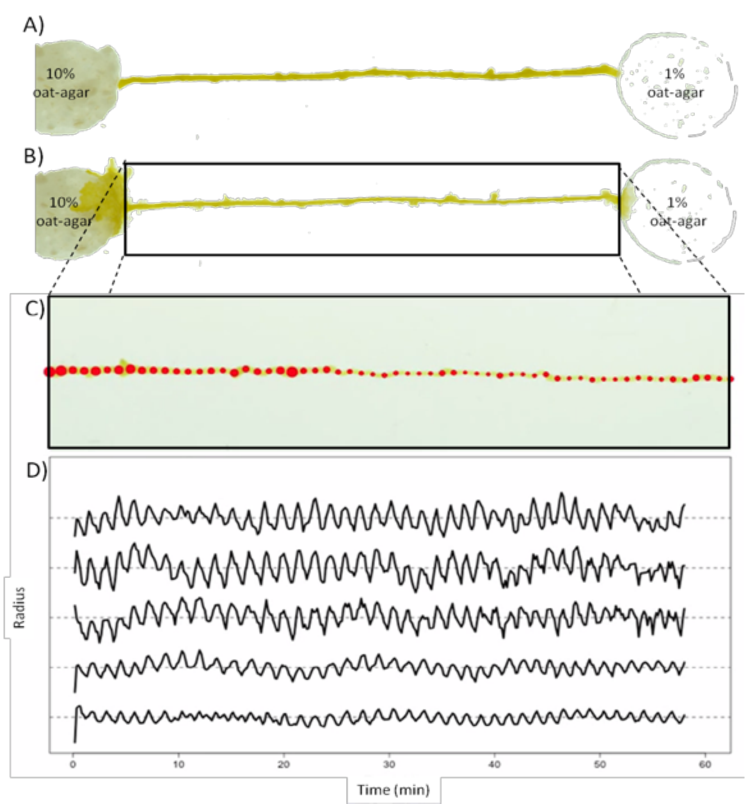

Figure 3: A) Tube-shaped slime mold cell of 3cm length in contact at its ends with a 10% oat-agar food disk and a 1% oat-agar food disk (experiment performed at PI Garnier's lab). **B)** The same cell an hour later, note that the biomass moved towards the more concentrated food disk. **C)** Example of the analysis performed by the custom shape-tracking software developed by PI Garnier – each circle fits within the boundaries of the cell at its location along the tubule; its radius thus follows the local dynamics of the width of the tubule. **D)** Waveforms displaying the oscillations of the diameter of the circles (and of the tubule) over 1 hour and at 5 locations along the length of the cell. Top curves correspond to points closer to the 10% food disk and bottom ones to points closer to the 1% food disk. Regions of the tubule closer to the higher concentration food source display an oscillation pattern that is different from that observed in areas closer to the lower concentration food source.

3.1.1 Automated detection and tracking of shape dynamics

Thin slime mold cells will be placed under conditions that stimulate their dynamics. These can range from simple growth on various substrates and nutritional environments to organism-level tasks such as choosing between two food sources by placing two oat-agar food disks in contact with either end of the cell (see Fig. 3A for an example of an experiment performed at PI Garnier's lab). Experiments on an agar dish will be placed on a LED panel with a 610nm long-wave pass filter. In previous experiments [11] it has been shown that PP does not respond to wavelengths of light that pass through this filter, whereas unfiltered light may induce a photophobic response. The transmitted light creates a strong contrast and highly defined cell edge that can be easily detected by the macro-equipped camera situated above the agar dish. This camera will be set to take time lapse images every second for one hour at high resolution and magnification. Over the course of the hour, the slime mold's choice will be visible by the higher amount of biomass moved onto one of the two food choices to digest it (Fig 3B). The amount of biomass in a region of the cell can be monitored experimentally by the amount of light penetrating the cell – thicker regions transmit less light. More importantly, the time lapse images taken during the decision will allow us to analyze the connection between local oscillations, organism-wide CD, and the decision-making process.

Oscillations will be measured using custom computer vision software - already developed by PI Garnier - that automatically detects the edges of the cell. For each time-lapse image, the program places a number of circles (chosen by the user; see Fig. 3C where 60 circles were used) at equal distances along the cell's length, and adjusts their radii to fit within the boundaries of the cell. The radius of each circle thus corresponds to the thickness of the cell at that location, and the temporal dynamics of each radius shows the local oscillation pattern of the cell (the dynamics of 5 of these radii distributed along the cell's length are displayed in Fig. 3D). We will study the relationship between global and local membrane oscillation patterns in terms of amplitude, frequency, and synchronicity.

3.1.2 Detection of internal flows

We will measure internal flows of the cytoplasm under various conditions, stimuli, and corresponding CD of the organism. These flows, which can attain speeds of over 1mms^{-1} [39], becomes visible at 4x magnification due to the transit of nuclei and granular vacuoles propelled by the flow. We will record these flows at specific points along the slime mold tubule as it makes a decision between two food sources of different quality, using a Nikon E400 trinocular microscope equipped with a Nikon D800 DSLR with AFS Micro Nikkor 105mm Macro lens. The videos will be analyzed using automated software based on optical flow [40]. This technique for measuring PP's internal flow has been tested successfully by PI Garnier and collaborator Reid for a separate project.

3.1.3 Measurement of surface forces

We will use traction force microscopy to measure the forces that the PP cells exert on the substrate as they move. PP cells will be placed on a soft polyacrylamide gel substrate with embedded fluorescent beads. The displacement of the beads within the substrate as the cells moves near to them, in conjunction with calculations of the stiffness of the substrate using its Young's modulus, will be used to determine the direction and magnitude of the forces exerted by the PP cells [41,42]. This technique is currently being developed and tested by PI Garnier in collaboration with Professor Gregory Weber (Department of Biological Sciences, Rutgers-Newark) for a separate project.

3.1.4 Direct mechanical stimulation

We will attach poly-L-lysine-coated magnetic beads to the cell membrane and induce oscillations in membrane diameter using a pulsed magnetic field from an electromagnet. This approach will allow us to exert localized forces over the membrane in a highly controlled way and *in vivo*, offering a subtle and not strongly invasive technique for inducing artificial membrane oscillations. As shown in Fig. 4, our pilot experiments have succeeded in attaching these beads to the slime mold membrane.

We may also explore, as an additional (and more invasive) method of direct mechanical stimulation, the possibility of using micropumps and microsyringes to manipulate the local volume of the slime mold by directly injecting cytoplasm (obtained from the same culture) in a localized region.

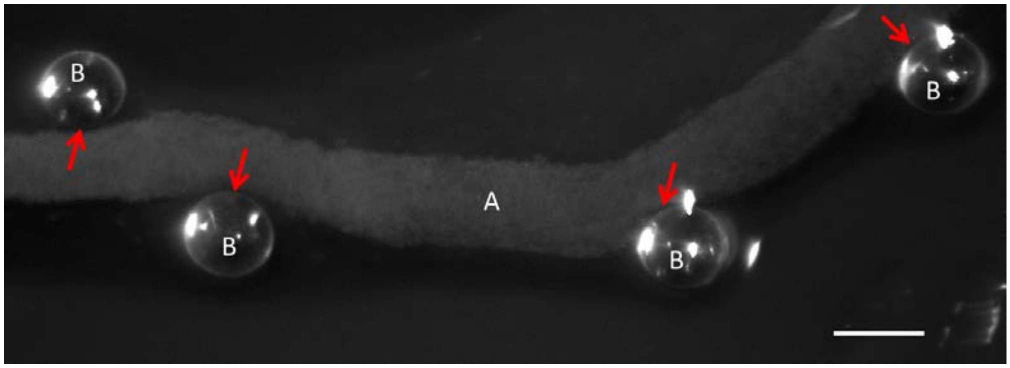

Figure 4: Pilot experiment showing a PP tubule (A) with magnetic beads (B) attached to the cell membrane. Attachment points are marked by red arrows. As the beads are displaced by an applied magnetic field, they deform the membrane in a controlled manner. The white scale bar represents 100µm.

3.1.5 Stimulation by light

We will shine light of different intensities on one region of the tubule in order to also induce cell response through sensory pathways and active biological machinery, instead of the direct manipulation induced by the mechanical stimulation approach in Section 3.1.4. (Light is known to have an inhibitory effect on membrane oscillations [43].) By comparing the effect of these different sources of stimuli, we will be able to differentiate biochemical reaction mechanisms from active or passive mechanical responses.

3.1.6 Boundary conditions, confinement, and behavior control

A simple yet powerful approach that we will use to explore the PP system will be based on our ability to constrain and control its structure and dynamics by imposing boundary conditions and/or external stimuli on the plasmodium.

The choice of boundary conditions is expected to strongly affect the resulting PP dynamics, since this is often the case in viscoelastic systems. By considering a broad variety of boundary conditions, we will be able to better investigate and manipulate the system response. A number of boundary conditions can be easily implemented by using a 3D printer to create specific confining environments for the plasmodium. A different type of boundary condition can be imposed by placing an acetate sheet with different shapes cut from it on top of an agar substrate. This will produce a moist agar surface with a specific boundary shape, surrounded by a dry acetate surface that is repellent to the slime mold. Since PP naturally spreads out within a bounded region in search of food, we will be able to produce plasmodia of different shapes and analyze their dynamics under different spatial constraints. Furthermore, since plasmodia can be grown in water, we will also be able to generate 3D geometries and to introduce other types of free boundary conditions, such as the water-air interface.

Our ability to place slime molds under different conditions by using physical constraints, as described above, will be combined with the use of various approaches for achieving specific PP dynamics through behavior control. Such control can be attained through stimuli that produce attraction or repulsion at the organismal level (e.g. food sources or certain salts [38,44–46]), by changing the environment (e.g. by adding chemicals to the substrate) or by directly affecting the local cytoplasmic activity (e.g. using different temperature ranges [47][48] or injecting chemicals into the PP). Furthermore, gravity provides us with an additional way to control PP, since this organism exhibits a preference for moving downwards [49]. In sum, our approach to behavioral control will take advantage of the wealth of chemotactic, phototactic, thermotactic and gravitactic responses known to occur in PP.

3.1.7 Fusion of cells induced to be in different states of CD

Using the manipulation techniques outlined above (3.1.4–3.1.6), we will fuse two PP cells, each induced to be in a different state of CD, such as having different oscillation periods or phases. We will then observe how the newly combined system reconfigures itself to achieve a single state of CD, using the measurement techniques described in (3.1.1-–3.1.3). The results of these experiments will be compared to theoretical predictions coming from the well-studied generic dynamics of coupled oscillators [50,51] and from our detailed numerical and analytical models (see Section 3.2 below).

3.2. Theoretical Framework

Our theoretical analyses will aim to relate the underlying self-organizing physical processes to the organism's evolved ability to display complex behavior. We will focus especially on tightly integrating the experimental setup with analytical and numerical modeling approaches for PP. Much work in both the physics and developmental biology literature has focused on generic mathematical mechanisms of pattern formation (Turing instability, KPZ equation, etc.), to the detriment of a more precise understanding of underlying physical dynamics and, in particular, of the role of local elastic couplings and of boundary conditions and geometry.

We will follow a three-pronged approach. Firstly, the active gel formalism, which was developed mainly for individual microscopic cells and is based on a continuous field that obeys known partial differential equations (PDEs) [15], is clearly appropriate for a macroscopic unicellular organism such as PP. We will extend this formalism to include the experimental geometries (e.g. tube-like shapes or with specific boundary conditions), and the interplay between sol and gel parts of the plasmodium. Given that spontaneous oscillations of the plasmodium are reminiscent of the chemical oscillations present during certain developmental stages, such as those occurring during the segmentation process in insects [52], we will integrate a chemical clock that mimics these types of dynamics if appropriate. Secondly, two of us have made progress in understanding the type of structures that can result from the coupling of activity and elasticity in particle-based systems. Our analysis of the role of inverse energy cascades and elastic low-frequency modes in this context (see 3.2.2 below) is relevant to PP, where the organism's geometry and boundary conditions will determine the structure of these modes. We will continue to pursue these investigations in the context of this specific system by developing numerical, particle based models of PP, which will be easily applicable to a range of geometries, boundary conditions, and perturbation techniques. Lastly, we will take advantage of the existing literature on mathematical processes that lead to nonequilibrium pattern formation and self-organization to systematically investigate the potential role of each one of these in the emergence of coherent structures and CD in PP.

We note that various models have been proposed to describe the dynamics of the plasmodium, mostly based on coupled oscillator dynamics in which local biological oscillators are linked mechanically through the gel elasticity and sol pressure transfer [53]. However, none of these models have been fully tested experimentally or attempted to link the emergent CD that results from underlying physical mechanisms to the various sophisticated organism-wide behaviors that PP can display, which range from attraction to specific stimuli [54] to finding the shortest path between food sources in a labyrinth [35].

3.2.1 Continuum description of active viscoelastic systems

The type of active viscoelastic behavior exhibited by PP, including its complex rheological properties, resembles those of in-vitro actomyosin gels and individual cells, and also tissue growth dynamics encountered in developmental biology. The first two have been well studied using recently developed active matter theory [3]. Conceptually related models also exist for tissues [2,55,56]. However, there are currently no models specifically for *P. polycephalum*. We will extend active gel theory [15] to describe these rheological properties and the active plasmodium dynamics, including its spontaneous oscillations.

Existing continuum active models (i.e. based on partial differential equations) start from symmetry considerations and conservation laws. They generally assume liquid behavior for the gel at long time-scales. In an active fluid that conserves mass and experiences both active and passive stresses, the starting point is an adapted Navier-Stokes equation,

$$\rho\left(\partial_t + \vec{v}.\vec{\nabla}\right)\vec{v} = -\vec{\nabla}(P_0 + P_{act}) + \vec{\nabla}.(\overleftrightarrow{\sigma}_0 + \overleftrightarrow{\sigma}_{act}),$$

where P is the pressure and $\overleftrightarrow{\sigma}$ is the traceless part of the stress tensor, both of which have been divided into their active and passive components. Active gel theory then derives both descriptions of the active and passive parts of the pressure and stress, as well as a closure relation linking, for example, the local polarization (modeled as a slow variable) back to the density. The symmetries of the active interaction, be they polar, nematic or purely contractile, strongly influence the observed patterns of active motion. For example, patterns of rotating vortices are produced by active polar filaments, but not by other interactions. Additionally, boundary conditions also determine the resulting dynamics, as observed when modeling the contractile ring during cell division [57] or defect dynamics on nematic active droplets [8].

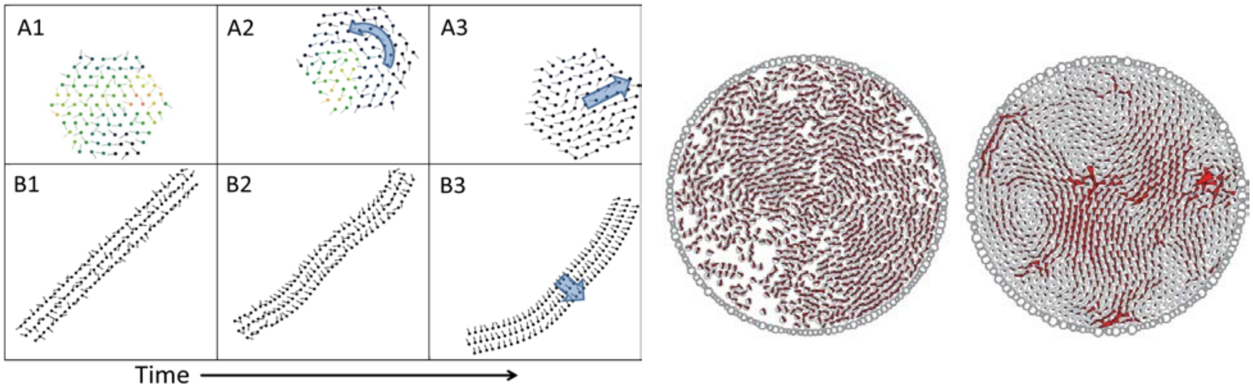

Figure 3. Simulations of the Active Elastic (left) and Active Jamming (right) models. Coherent dynamics spontaneously emerge in groups of elastically-linked self-propelled components. Left panels: Agents initially advance in random directions (frames A1/B1). Over time (A2/B2–A3/B3), coherent dynamics develop, deforming and translating the group as a whole. Right panels: Snapshots of the liquid (left) and jammed (right) phases, with red arrows representing the instantaneous velocity field. In the jammed phase, coherent large-scale oscillations emerge, as visible in the coherent arrow structure.

When describing individual PP tubules, our active gel model will take into account the cylindrical symmetry of the active actomyosin components and the elastic coupling between neighboring segments of the tubule. The sol flowing through the tubule is an incompressible liquid and will contribute to a hydrostatic pressure counteracting the active contractility of the tube. The proposed experimental perturbations will allow us to directly probe the active elastic response of the tubule. Using a Green's function formalism, we will make corresponding quantitative predictions deriving from our model equations.

3.2.2 Agent-based models

In addition to the continuum descriptions above, two of us have recently pioneered the study of agent-based models of elastically-coupled active components [4,58]. Huepe et al. and Henkes et al. independently developed the Active Elastic (AE) and Active Jamming (AJ) models, respectively, in which groups of self-propelled agents that advance over a substrate (due to their internal driving energy) self-organize into CD through a mechanism that combines activity and elasticity. Although the active dynamics of PP is of a different nature (it is driven by extension and contraction forces that produce internal viscoelastic flows, rather than by self-propulsion), both of these works show a novel and generic reverse energy cascade mechanism that can lead to CD, which we expect to also play a role in the case of PP. We will thus briefly describe here these models and mechanism.

The models describe groups of self-propelled agents interacting through short ranged forces (double-sided harmonic springs in the AE case; one sided in the AJ case). In contrast to the continuum viscoelastic descriptions discussed above, which present liquid behavior at long time scales, these models effectively map to a spring-mass model of an elastic sheet. Both models can be summarized as follows. We consider a system of N agents on a two-dimensional plane, with positions $\vec{r}_i$ and orientations $\hat{n}_i = (\cos\theta_i, \sin\theta_i)$ that evolve according to

$$\dot{\vec{r}}_i = v_0 \hat{n}_i + \mu \vec{F}_i$$
$$\dot{\theta}_i = \frac{1}{\tau}\,\text{alignment} + \eta_i.$$

These equations are fully overdamped, i.e. they neglect all inertial components, as is appropriate for micron-sized cells or particles moving dissipatively over a substrate. Here, v_0 is the internally generated active self-propulsion speed that injects energy at the individual particle level. $\vec{F}_i$ is the total force on particle i by its neighbors through the elastic pair forces (AJ case), or its projection onto the orientation vector $\hat{n}_i$ (AE case) [59,60]. Noise can be added to each agent's position and/or orientation. The second equation governs the relaxation of the orientation angle towards a value determined by its local environment. In the AJ case, the 'alignment' term is $\vec{F}_i \cdot \hat{n}_i^{\perp}$: the force components orthogonal to the orientation. In the second case, it is the angular difference between the orientation vector θ_i and the direction of the actual velocity θ_i^v. In both cases, it is crucial that the orientation couples back to the actual forces generated by the elastic interactions, thus enabling the reverse energy cascade described in more detail further below.

The left panel of Fig. 3 displays the dynamics of two different self-propelled elastic sheets described by the AE model; from the initial condition where agents are randomly oriented (A1/B1) to coherent rotational (A2), vibrational (B2), and translational (A3/B3) motion. For random initial orientations and low enough noise, the system organizes into larger and larger regions of coherent motion, eventually reaching the largest CD allowed by its boundary conditions. The right panel of Fig. 3 shows the dynamics of the AJ model for two different mean particle densities. This model was designed to describe a group of self-propelled particles with soft repulsive interactions, and was motivated by *in vitro* experiments on confluent monolayers of migratory epithelial and endothelial cells. Since particles here are confined within a circular region, no collective displacement is possible, but instead CD is given by coherent large-scale oscillations that span the whole system when jammed. The rightmost panel shows a state that reached CD, in which the instantaneous velocity field (red arrows) displays a coherent structure.

The AE and AJ models both display a novel mechanism for CD, which is based on an inverse energy cascade that drives the self-propulsion energy injected by each agent to lower and lower elastic modes, and thus to larger and larger regions of coherent motion in the homogeneous systems considered here. We expect a similar mechanism to emerge in other systems that combine activity and elasticity, including the PP. We will use the APCS (Active Particles on Curved Surfaces) code [6] jointly developed by PI Henkes and collaborator Sknepnek to explore this and other potential mechanisms for CD in simulations under conditions that mimic the structure and dynamics of PP. For example, Fig. 4 shows a preliminary 3D calculation of active components (here following AJ dynamics) in a tubule-like geometry with fixed ends. Starting from random initial conditions, CD emerges as agents spontaneously self-organize into a low mode with three segments of coherent axial rotation. We will study the potential role of this and other mechanisms for CD in PP by modifying these simulations to include extension-contraction dynamics and features particular to PP. A significant advantage of agent-based modeling is that it will allow us to easily develop multiple different and increasingly complex simulations of PP-like dynamics. These can explore new geometries and boundary conditions, multiple regions with different and changing active viscoelastic properties, and biochemical signaling by simply modifying particle features and inter-particle interactions (see our recent work on a sphere [6]). For example, we can simulate tubule-like dynamics by modeling sol as adhering particles on a cylinder and gel as soft, frictionless particles moving inside as a fluid.

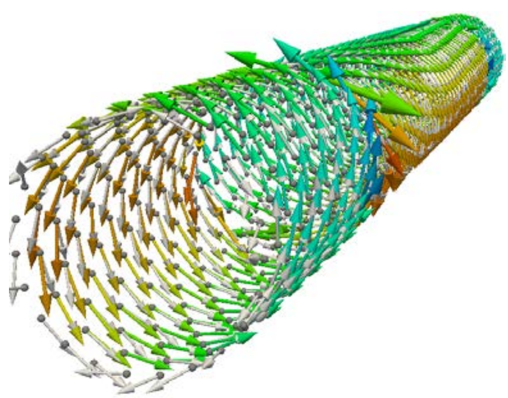

Figure 4: Active jamming dynamics constrained to move on the surface of a tube, inspired by the geometry of a PP tubule, simulated using the APCS code [6]. Particles spiral here in three counter-rotating sections separated by defect lines. Due to the fixed ends of the tube, the softest direction of motion (lowest energy mode) is around the tube. Velocity vectors have lengths proportional to their magnitude and are colored according to their direction. The particle orientation vectors $\hat{n}_i$ are displayed in gray. Particles here fully cover the tube surface (with density Φ=1) and are not drawn to scale.

3.2.3 Nonequilibrium pattern formation and self-organization

In addition to the mechanism leading to CD described above, various other self-organizing and pattern-formation mechanisms have been previously studied in the context of nonequilibrium dynamics.

In the developmental biology literature, several mechanisms for pattern formation in a biological context have been investigated in detail (see [55] for a review). Reaction-diffusion models are adapted from a similar ansatz as active gel theory, but include the diffusion of morphogens produced by individual cells within a gene regulatory network. Such models describe a variety of patterns emerging in development, such as the stable chemical oscillator responsible for segmentation in the drosophila embryo (the segmentation clock) [52], or the Turing patterns evident in the skin and fur colorings of a variety of animals [61,62]. Several types of cell-based mechanics (e.g. sorting due to differential adhesion, sedimentation gradients or actual motion of polarized cells) have also been implicated in the early stages of embryo formation, such as formation of the blastula and gastrulation [55,63,64].

In a different species of slime mold, *Dictyostelium discoideum*, individual crawling amoeboid cells react to chemical signals following a Belouszhov-Zhabotinsky (BZ) reaction-diffusion pattern. The resulting spiral chemical waves lead to the cells adapting a convergent motion and forming a proto-metazoic 'slug' that can move on its own [10] (again exploiting BZ-type dynamics) and eventually form the fruiting bodies [9] and spores necessary for sexual reproduction.

It is unknown whether chemical gradients play a role in developing and maintaining the rhythmic oscillations of PP. As we develop and integrate both elasticity-based and reaction-diffusion based models of PP dynamics, they will lead to significantly different predictions, in particular as a function of boundary conditions. This will allow us to construct experimental tests that determine the role of chemical and mechanical processes in PP, if these change under different conditions, and if they act in combination,

We note that the different mechanisms described here produce regions of CD with characteristic scales and features that will depend on the underlying physical parameters. We will take advantage of this relationship to determine which mechanisms are behind the observed CD, by carrying out experiments that perturb the cytoplasm of the PP plasmodia (i.e. the parameters of the medium). We also note that the energy cascade mechanism for CD described in subsection 3.2.2 is fundamentally different from the mechanisms presented above. Indeed, boundary conditions determine the emerging features that result from the former whereas they do not play any role the latter.

4. Integrated Experimental and Theoretical Research Plan

We will build on the experimental and theoretical approaches described above to achieve the three Aims listed in the Project Overview, which are used to structure the following Research Plan.

Aim 1: Build realistic biophysical models of *P. polycephalum* dynamics by combining specifically tailored experiments with recent progress in the theory of active matter

This Aim will be achieved by carrying out multiple parallel activities. Experimentally, we will use the techniques described in Section 3.1 to monitor the local and global PP dynamics in detail. We will prepare PP samples with different physical and biological properties. These will include different cell sizes (by adding more slime mold mass), multiple cell shapes with specific boundary condition properties (such as circular cells and star-shaped cells) different substrate media (such as sloping agar, or hardened/softened agar), and different cell states (such as starved vs fed plasmodia). By growing slime mold plasmodia on the surface of water, we will be able to separate sections from the parent plasmodium and transfer them to individual agar plates. These sections self-organize to become new, tube-shaped individual cells within minutes [37]. We will place them in different experimental setups to address specific questions.

Our ability to introduce localized forces and internal flows while monitoring the system response will allow us to carry out detailed rheology experiments under a variety of conditions. We will explore how the viscoelastic response depends on PP's internal composition and active biological processes by extending the usual rheological analyses to consider inhomogeneous internal structures, changes of biological states, and the localized injection of energy in active matter. We will thus characterize the system rheology as a function of biological conditions.

From a theoretical perspective, we will seek to match these empirical results using the two different modelling approaches described in Section 3.2: (i) continuum descriptions of the active viscoelastic matter inside PP and (ii) agent-based computer simulations of active viscoelastic components that can represent complex nonhomogeneous microscopic dynamics. For the first approach, we will set up PP samples under conditions where a significant region of homogeneous material can be probed independently and with well-defined boundary conditions. For example, we will apply controlled amounts of force to the plasmodia through direct mechanical manipulation (using the techniques described in 3.1.4 and others) and measure the corresponding mechanical response. This will allow us to explicitly compare stress-strain relations and dynamical properties predicted by the continuum equations with experimental results on the PP system. For the second approach, we will first continue to develop the type of microscopic model simulations shown in Fig. 4 to include active contractile elements that properly describe the local PP dynamics. This approach will allow us to model complex, non-homogeneous internal structures and interactions, such as combinations of fluidized and non-fluidized material, couplings between biochemical and viscoelastic dynamics, and dynamical boundary conditions. It will also help us explore potential mechanisms for self-organization that would be missed by continuum descriptions, since they require reductionist boundary conditions and continuous field approximations. We will take advantage of local external collaborators near PI Henkes to help carry out these analyses, including several experts on non-linear dynamics at the University of Aberdeen, such as Prof. Antonio Politi. These numerical calculations will be carried out using the three-dimensional simulation code APCS, developed by Dr. Sknepnek and Dr. Henkes (see commitment letter in supplementary material).

In order to achieve this aim, we will use continuum and agent-based approaches to formulate a combination of general and detailed models of the PP system. General models will aim to capture material properties of different homogeneous sections within the plasmodium and to identify generic emergent dynamics. Detailed models will study the effects on PP dynamics of specific features such as tubule geometry and boundary conditions. They will include particle-based simulations of the whole organism, building on the existing APCS code. Close interactions between theory and experiments will allow us to test theoretical predictions using new experimental setups. We will also set up directly comparable numerical and laboratory experiments under equivalent conditions, in order to validate our theoretical approaches, assess their ability to predict system dynamics, and find model parameters.

Aim 2: Analyze theoretically and experimentally the various mechanisms for pattern formation and self-organization that could lead to CD in *P. polycephalum*

To achieve this aim, we will first survey and catalogue the conditions that generate CD and pattern formation in specifically designed experimental setups and in the models built for Aim 1. We will take into consideration all potential processes for CD and pattern formation described in Section 3.2. We will then analyze their dynamics and underlying mechanisms. Our objective is to go beyond the usual analysis of qualitative resemblances between experimental patterns and mathematical patterns produced by phase equations. We will seek instead to discriminate between potential underlying mechanisms by taking advantage of our ability to experimentally impose boundary conditions, internal flows, and plasmodium properties to induce a collection of patterns and CDs that can be compared with theoretical predictions.

We will seek to generate one- and two-dimensional patterns on experimental preparations where PP forms tubules or covers extended surfaces, by using the plasmodium's self-organizing capabilities, external periodic forcing, etc. These patterns will be generated while placing PP under a number of different conditions. We will introduce perturbations, including substrate surface, mechanical, temperature [48] or light [65] induced periodic stimuli, as described in Section 3.1, which could potentially alter the nature of the emerging patterns and CD. We will pay special attention to spontaneous oscillations of the plasmodium, which have been shown to occur both in active gels [3] and active solid theories [4,58] and have been linked in the latter to low-energy elastic modes (phonons) that produce system-wide CD.

We will seek to distinguish two classes of pattern formation mechanisms: one where characteristic structures are determined only by the parameters of the medium and one where they depend strongly on the boundary conditions. We will explore which type of mechanisms could be most beneficial for achieving desirable organism-level behavior under different conditions.

Aim 3: Link the underlying dynamics of the function and behavior of *P. polycephalum* to its known ability to solve survival challenges, including optimization and decision making.

We will examine if the patterns and structures that naturally emerge in theoretical and experimental settings can be related to self-organized biological processes that are beneficial for organism survival, analyzing their potential evolutionary role through simulations. Given PP's ability to control its shape to achieve desirable behavior despite its lack of centralized control, we expect pattern formation to be part of the mechanisms used by PP to develop organism-level CD.

More specifically, we will prepare PP cells to carry out tasks that are known to require organism-level responses, such as choosing between two food sources of differing quality by placing two oat-agar food disks in contact with either end of the cell (Fig 3A), while analyzing their detailed dynamics. We will initially consider plasmodial tubules that result from small slime mold cells (2cm in length), due to their simpler geometry, which is quasi one-dimensional. Using time-lapse macrophotography taken every second for one hour at high resolution and magnification, together with other experimental approaches described in Section 4.1, we will measure the amplitude and frequency of the membrane oscillations and internal flows, at different locations along the tubule. We will link local and global dynamics by measuring how natural oscillation patterns, as well as artificial oscillations that we will impose at one or more locations (in and off phase), affect distant regions of the cell. Our objective is to link how localized CD driven by biophysical dynamics develops into organism-level behavior that favors survival.

We will benefit in these investigations from our ability to carry out the experiments above under a variety of conditions (using the approaches described in Section 3.1). These include: (i) changing the concentration ratio between the two food sources, (ii) influencing surface membrane oscillation using attached magnetic beads, and (iii) inducing an external repellent stimulus using a varying light field.

One of our starting points will be to reproduce the results in [48] where the food source chosen by a PP cell could be controlled by stimulating PP with specific oscillation patterns. We will then attempt to understand this decision-making process as a physical process, using the results obtained under Aims 1 and 2 to unveil the connection between the physical CD and organismal behavior. We will take a similar approach to explain the ability of PP to find the shortest path between food sources.

Ultimately, we seek to be able to manipulate PP as a controllable excitable medium, even engineering new dynamics or function through direct experimental intervention of the internal flows and/or boundary conditions. By applying control theory approaches, we will attempt to implement automatic control loops that link the various types of stimulation and measurement techniques described in this proposal to produce desired CD. We will also attempt to implement logical computations on PP [66–69] through phenomenological approached. Both efforts will also produce new insights into the PP system.

5. Broader Impacts

5.1. Interdisciplinary Training

A key outcome of the proposed work will be the interdisciplinary training of postdoctoral researchers, graduate and undergraduate students – even the PIs. Such training is fundamental in modern biological sciences to tackle the increasing complexity of the studied systems. To achieve this training, all team members will participate in research outside their primary discipline. The biology members will actively take part in the design and analysis of the mathematical models, based on their knowledge of *P. polycephalum* behavior. The biology graduate research assistant (GRA) will also be trained in designing and programming models of complex biological processes. The mathematics and physics members will actively contribute to the design of the experiments with *P. polycephalum*, using the models to propose new testable predictions. The 2 postdoctoral researchers (PRs) will also spend several weeks in PI Garnier's lab to become familiarized with the studied organism and assist in the execution of the experiments. We expect that the projects will require an integrated research approach, which will further develop these synergies as members will be driven to close interactions by the project's combined theoretical and experimental requirements. This project will also allow us to provide interdisciplinary research experience to undergraduate students, who would not normally be exposed to it. The US members will use well-established programs for summer undergraduate research experience (REU) to recruit students and specifically include them in cross-group research efforts that expose them to multiple universities. At the University of Aberdeen, Henkes will recruit several undergraduate students for their final year honours research project and involve them directly in the ongoing research effort. Huepe will participate in summer programs at Northwestern U (for at least two summers during the project period) that allow undergraduate students to get involved in short-term research efforts, directly mentoring students and involving them in research related to the project. Henkes continuously introduces active matter models in the undergraduate physics programming course (UoA PX3017). In addition, Garnier teaches formal classes on interdisciplinary topics at the undergraduate and graduate level (NJIT BIOL 337/698). He will develop a module based on the role of physical mechanisms in the emergence of complex organized behaviors to include in these courses, which will also be freely available on the web.

5.2. Public Outreach

The slime mold *P. polycephalum* is a particularly good system to involve the public and students in hands-on scientific projects. Its acquisition and maintenance costs are low, its handling is safe and easy, it does not raise ethical questions, and it is possible to perform multiple experiments simultaneously with it. Science teachers in secondary education commonly use it for their classes and multiple research kits are commercially available. We will exploit this for public outreach by creating "hands-on" demonstrations that can be run by all three PI groups and easily be taken to public venues and schools at all levels. PP can be used to illustrate areas as diverse as cell locomotion, the material and dynamical properties of cell gels, viscoelastic dynamics, non-Newtonian fluids, the emergence of structure in living systems, decentralized decision-making, energy flow dynamics in a simple organism, self-organization, etc. We will develop a simplified version of our experimental setup using affordable material and equipment (e.g., webcams and notebook computers instead of DSLR cameras and desktop PCs), that will allow outreach participants to perform and automatically record experiments over 24 to 48 hours, which is the usual timescale for experiments with *P. polycephalum*. This simplified experimental setup will be made part of outreach travel kits (which fit in a suitcase and are easy to use by any group members) that will be used at the three participating institutions. The building instructions of the simplified setup and the accompanying software source code will be made available online under Creative Commons and GNU GPL licenses to facilitate its use by science teachers. This outcome will provide us with interactive hands-on tools that are known to be effective at creating engagement amongst participants of all ages. All of our labs have multiple opportunities throughout the year to demonstrate at public science venues, museums, and to visiting K-12 students, especially minority groups. We will actively seek out opportunities to give public lectures and demonstrations on this research in front of non-specialist audiences, for example in the café scientifique lecture series in Aberdeen and other locations in the UK.

5.3. Recruiting and Retention of Minorities

The three PIs are committed to increasing participation of minorities and women in science. They will continue to actively seek out female students and postdoctoral researchers, which is particularly relevant since women are largely underrepresented in mathematically intensive fields. Although biology at the undergraduate level is closer to parity, much remains to be done at higher levels of academia to retain and support women. Increasing the access of minorities to higher education and to science related jobs is also a constant concern for the three PIs. PI Garnier's lab belongs to the Federated Department of Biology at NJIT and Rutgers-Newark. U.S. News and World Report have ranked both campuses amongst the most diverse national universities in the USA: 1st for Rutgers-Newark and 6th for NJIT [70]. His team includes a majority of students from underrepresented groups and he has a proven record of mentoring female students. PI Henkes currently co-supervises one female doctoral student with an undergraduate degree in biology, and focuses especially on providing the required mathematical and programming training. She is a member of the COPS (College of Physical Sciences) Equality and Diversity committee of the University of Aberdeen, which is assisting the Athena Swan Charter re-submission of the University. As a group we are committed to cross-mentoring minority and women graduate students and postdocs supported by this grant. In particular we will exploit our connection between biology and mathematics/physics, to expose more minority and women biology students to mathematical thinking, and to recruit more women in mathematical and computational biology.

5.4. Other Activities

Our groups have strong track records on education and public outreach, and while we have outlined several planned activities here, this grant could also provide funding to support opportunistic activities that continually arise in our settings. Garnier is especially interested in the public perception of science. In addition to regular public lectures for non-specialist audiences, he is very active in the online science community covering science results broadly (1,200+ followers on Twitter; 23,000+ on Google+). He also develops open source, interactive web application for students to discover and manipulate classic models of collective behaviors (https://github.com/sjmgarnier/Shiny). Henkes and Sknepnek will produce a web interface to the APCS code, aimed at the general public. Huepe is a professional musician involved in electronic music and in various art/science projects, and will incorporate concepts, images, and data from the project in his musical creations and performances. Such projects will aim to generate strong press coverage and reach a broad nonscientific audience often not engaged by standard outreach activities.

Both Garnier and Huepe have a track record of press and media interactions and cultivate relationships with journalists, which will facilitate the dissemination of project results to the broader public. In the past, this has led to news features in Wired Magazine, National Geographic, BBC News, BBC Nature, The Wall Street Journal, The Los Angeles Times, The Guardian, Scientific American and other high-profile national and international media.

6. Project Management and Coordination

Garnier, Henkes and Huepe will be the 3 senior researchers in charge of the project. We also seek funds for one graduate research assistant (GRA, based at NJIT with PI Garnier) and two postdoctoral researchers (PR). One will be based at the University of Aberdeen with PI Henkes for the full term of the project and the other one based at Northwestern University with PI Huepe for two years. The work proposed in this project will comprise the bulk of the GRA's PhD research. The GRA and the two PRs will be co-supervised by the three PIs, thus reinforcing the interdisciplinary interactions between our research teams. In addition to this core team, the project will benefit from the expertise of two external collaborators: Dr. Christopher Reid (NJIT, specialist of *P. polycephalum* behavior) and Dr. Rastko Sknepnek (University of Dundee, specialist of numerical simulations and analysis).

This collaborative project resulted from a history of scientific interactions between the three PIs, based in common interests in their parallel and complementary fields of expertise. Garnier and Huepe have been involved in discussions on the role of self-organization and physical processes in living systems for over four years, since they started communicating as a result of their common collaboration with Prof. Iain Couzin (Princeton U). Henkes and Huepe have long shared an interest on the role of novel physical mechanisms, especially resulting from active matter dynamics, on complex, nonequilibrium, and living systems. They have had regular discussions since 2011, when they developed parallel models of active components with elastic interactions. As participants of the recent KITP workshop *Active Matter: Cytoskeleton, Cells, Tissues and Flocks*, Henkes and Huepe discussed combining their common interest in active viscoelastic dynamics and to connect it to concrete systems and relevant biological processes. The new PP experimental platform that was being implemented by Garnier came as the perfect system to link to their theoretical work and the *UK BBSRC-US NSF/BIO Lead Agency Pilot Opportunity* as the ideal mechanism for supporting this effort. The natural convergence of the PI's respective research programs and their common motivations will ensure successful project integration.

While the three PIs will be involved in all aspects of the project, each of them will oversee a different section of the proposed work. PI Garnier will organize and supervise all proposed experiments. He will mentor the GRA based at NJIT, performing the experiments and their analysis. He will collaborate with Dr. Reid to design and implement the experiments. PI Garnier will also be responsible for ensuring the successful completion of the outreach activities discussed in the broader impact section, with all team members taking part in these activities and having opportunities to be trained. PI Huepe will be responsible for coordinating the connection between experimental and theoretical results. His position as a Research Scientist allows him the flexibility to have regular visits with the groups of Garnier and Henkes, which will help keep the project cohesive. PI Huepe will mentor and supervise the PR based at Northwestern University, focusing on simple models and perspectives that capture the connection between the biophysical features of the system and emergent organismal behavior. He will also work with PI Henkes to perform analytical calculations and detailed numerical simulations. PI Henkes will focus on calculations and simulations of viscoelastic active matter systems and pattern formation processes that lead to organism-wide coherent dynamics and the emergence of biological structures. She will mentor and supervise the PR based at the University of Aberdeen, analyzing continuous (PDE-based) mathematical descriptions and detailed numerical models. She will interface with Dr. Sknepnek (based at the University of Dundee, about 1 hour south of Aberdeen) to implement detailed numerical simulations using their joint APCS code, within the framework of an existing NRP (Northern Research Partnership) collaborative grant. Henkes will also take advantage of the expertise at Aberdeen on pattern formation processes to explore a variety of mechanisms that could result in the observed dynamics.

Due to the international nature of this collaborative work, the project members will interact via videoconference at least twice a month to coordinate their research efforts. These virtual meetings will include research updates from the GRA and the two PRs. The US-based members will meet at least once every semester of the project, and the entire project team will meet at least once a year for milestone meetings, either in the US or in the UK. During these meetings, the overall research progress, coordination, prioritization, collaborations, and potential applications will be discussed, while upcoming challenges and opportunities will be identified and addressed.

In addition to these regular meetings, our proposed budget includes resources to enable the PRs to visit the other PIs' lab for several weeks each year. In particular, the PRs will be expected to use this opportunity to assist with the experimental work performed in Garnier's lab. Finally, further project cohesion will be guaranteed by PI Huepe, who is based in Chicago (and will thus be able to visit Garnier's lab often) while currently spending 2 to 4 months every year in Europe (at no cost to the project), which will allow him to also interact regularly in person with Henkes' research group.

All members of the project team will be encouraged to present their work at national and international meetings and the proposed budget includes funds for this purpose. As part of the graduate training program at NJIT, the GRA will also present his/her work at least once a year at the student-run Biological Sciences Department weekly Colloquium Seminar Series. The PRs will also present results locally, at least once a year, in seminar series at their host institutions.

7. Results from Prior NSF Support

Award PHY-0848755: Experimental and Theoretical Analysis of Collective Dynamics in Swarming Systems. Subaward PI: Cristián Huepe; Subaward amount: $194,100 (9/1/09-8/31/13) (main-award PI: Prof. I D Couzin, Princeton U.; total award: $543,472). This project studied collective motion, schooling and swarms experimentally and theoretically from a non-equilibrium statistical physics perspective.

Intellectual Merit: Theory (Huepe): developed detailed and idealized models to capture specific and universal aspects of swarm dynamics based on experimental results and theoretical analysis; introduced an adaptive-network description of collective motion; solved the inverse problem for small groups of fish, deducing the interactions between individuals from experimental data thus unveiling non-additive effects; extended standard swarming models to include variable speeds, visual interactions, etc.; characterized transition dynamics between states of collective motion; developed new approaches for understanding collective decision making in animal groups; discovered a novel elasticity-based self-organization mechanism for active systems that leads to collective motion even in the absence of explicit alignment. Experiments (Couzin): implemented fully controlled fish swarming experiments, tracking all individuals.

Broader Impacts: Huepe co-trained four graduate students and five postdoctoral researchers; organized the interdisciplinary art/science meetings *Arts, Humanities and Complex Networks* (within NetSci2012) and *Networks and Nonlinearities in the Musical Experience* (at the ZiF Center for Interdisciplinary Research, Germany), which received broad media coverage; participated in public lectures and a roundtable on *Physics of Music* at the Chicago Cultural Center; worked as a scientific consultant for the *eduMedia* educational website; developed control algorithms that were applied in swarm robotics engineering; gave keynote public presentations on music and complex systems in Koblenz (Germany) and Viña del Mar (Chile). Media coverage: Wired Magazine, National Geographic, Focus Online, MSNBC blog, etc**.,** and articles in a broad range of international press. (Main award PI, I. D. Couzin, also contributed to the project's Broader Impacts by leading CouzinLab, teaching at Princeton U, participating in advisory panels, and giving public lectures and media interviews.)

Publications (by C. Huepe): The project led to 12 peer-reviewed papers on various areas, including: experimental studies of collective behavior in schooling fish [71,72], adaptive network models of swarm dynamics [73]; decentralized control of swarm robotics and artificial life applications [74,75]; continuous theory of collective motion with variable speeds [76], network-based analyses of the opinion-formation models [77], criticality in living systems [78,79], self-organization in swarms and active elastic systems [58,80], and art/science interdisciplinary research on music and complex systems [81]. (This project also led to over 25 other publications by Couzin's group, including 2 in Science and 5 in PNAS)

Other products and activities: Huepe wrote a book chapter on 'Flocking and Music' for *Controls and Art* (Springer, 2014) [82]. He was also a member of the Advanced Study Group: *Statistical Physics of Collective Motion* at the Max Planck Institute for the Physics of Complex Systems (Dresden, Germany, 2011-2013). Data, models, and code resulting from the project are available at CouzinLab's and Huepe's websites: http://icouzin.princeton.edu/ and http://people.esam.northwestern.edu/~cristian/.

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