

Chapter 6

Accounting for the fundamental dissipative costs of gradient formations across space and time in motor-microtubule assemblies

This chapter describes joint work with Ana Duarte, Rob Phillips, and others in the Phillips lab. In this project in progress, GS has formulated theory, copiloted the data analysis pipelines, and cowritten the manuscript.

So far in our journey through this thesis, the values of measured energy expenditures we have discussed have not been explicitly space-dependent. Our empirical coordinates have been whole-cell basal metabolic rates, or the nonequilibrium driving force operating in a mean field gene-protein binding network understood as well mixed. Thankfully, however, biology is profoundly nonuniform. These patterns are a flavor of lifeiness that surely costs energy to durably sustain. Over the past years, colleagues in the Phillips laboratory (especially Ana Duarte, HJ Lee, and many others) have carefully built and ushered into use a fluorescent probe that can report on local concentrations of ATP in space in controllable

in vitro assays. Accordingly, these measurements afford the means to measure power density as a field variable that explicitly and meaningfully depends on space and time. Over the past years we have worked with these intrepid colleagues to make more robust the local inference of ATP from noisy biological data. Simultaneously we have enjoyed scaling up minimal but, to our knowledge, fresh theory that makes strong predictions for where and when power should be maximal, and from what specific physical origins power demands likely stem.

In this first of two chapters, we proceed through our draft findings of the quantitative phenomenology of gradients of ATP resolved while molecular motors conspire collectively to form patterned arrangements of microtubules. In the chapter that follows (Chapter 7), we spelunk through nascent theory that, one day, should converse with experiments.

6.1 Overview

Living matter produces a variety of beautiful spatiotemporal structures and patterns that are not present in their nonliving counterparts. Often, these ordered, non-equilibrium steady states are sustained through an influx of energy. Careful examination of when and where living systems direct energy consumption helps us understand the principles dictating such ordering and can motivate development of non-equilibrium theories. Here, we investigate the energetic cost of assembling an ordered aster from a disordered, uniform mixture of cytoskeletal microtubules and kinesin motors. Using a fluorescent ATP sensor, we perform a careful measurement of ATP consumption through space and time on an *in vitro* cytoskeletal network. Our experiments resolve the emergence of radial ATP gradients. We successfully predict how a given motor profile generates these ATP distributions using reaction-diffusion models in conjunction with finite element simulations. With our results, we compare the power per volume required by our

cytoskeletal networks with the known power per volume expenditure in cells. Evoking the direct quantification of energetic fluxes from our spatial measurements, unlocks future exploration of what steady states are accessible to cells and how the cytoskeleton drives spatial organization.

6.2 Introduction

One of the main drivers maintaining the rich patterns in living matter is a steady investment of energy. For example, the existence of morphogen gradients in developmental patterning is paid for through a steady flux of protein synthesis and degradation. Similarly, cytoskeletal-motor systems hydrolyze ATP and GTP to achieve processes ranging from intracellular transport, to cell motility, to chromosome segregation. Motivated by these processes, we were inspired to develop a physical understanding of the connection between energy fluxes and the emergence of biological order in space and time in the context of the particular example of microtubule-motor assemblies.

The energetic basis of the processes within living cells are based upon a few fundamental energy currencies, which can be thought of as a sort of biological batteries. This metaphor is useful because it reminds us that batteries are indifferent to the particulars of what they are wired up to - they can drive anything from the light in a flashlight to motorized toys. Biological processes are powered by several key biological batteries including membrane potentials, redox reactions and trinucleotide hydrolysis. Indeed, for the molecular motor driven reactions that power the structures of the cytoskeleton, ATP and GTP hydrolysis are central. Thus, we were curious about how ATP consumption in space and time drives the dynamics of structure formation.

Recently, it has become possible to measure the total energy consumption of both living

organisms and the molecular machinery that drive them [1, 2, 3, 4]. We aimed to complement these foundational studies by investigating the spatial distribution of energy consumption. In particular, here we report the visualization of spatial, ATP concentration gradients across cytoskeletal networks, giving insight into how structure, composition and morphology drive energy dissipation.

We use motor-microtubule assemblies as a highly controllable, tunable system due to their minimal components and self-organizing properties in vitro as well as their biological ubiquity. An abundance of work has established that connected dimeric motor domains can cross-link microtubules, creating ordered networks [5, 6, 7, 8]. To control the position, size and start of microtubule cross-linking, we optogenetically link motor proteins together as shown in Figure 6.1, as previously developed in our labs [8]. Motors harness energy to drag microtubules into ordered structures by hydrolyzing an ATP molecule for each step they take along the microtubule. We measure the energy consumed by the motors throughout space and time using a fluorescent, QUEEN based ATP probe [9] as shown in Figure 6.1.

In addition, to complement the measurements and to provide a framework for understanding them, we develop a reaction-diffusion equation that describes the emergent ATP spatiotemporal gradients and explore the implications of that model with finite element calculations. Combining our experimental and theoretical results, we determine that the measured network formation power is indeed many orders of magnitude greater than the theoretical power of equilibrium processes, and we demonstrate the novel value of spatial measurements by bounding energy dissipation with Jensen's inequality.

We note that the work presented in the remainder of the paper is written with the intention of providing the essential narrative structure in the paper itself. In parallel, the Supplementary Material is written with the aim of making every technical detail of how the experiments were done, how the data was analyzed, how conclusions were drawn and how

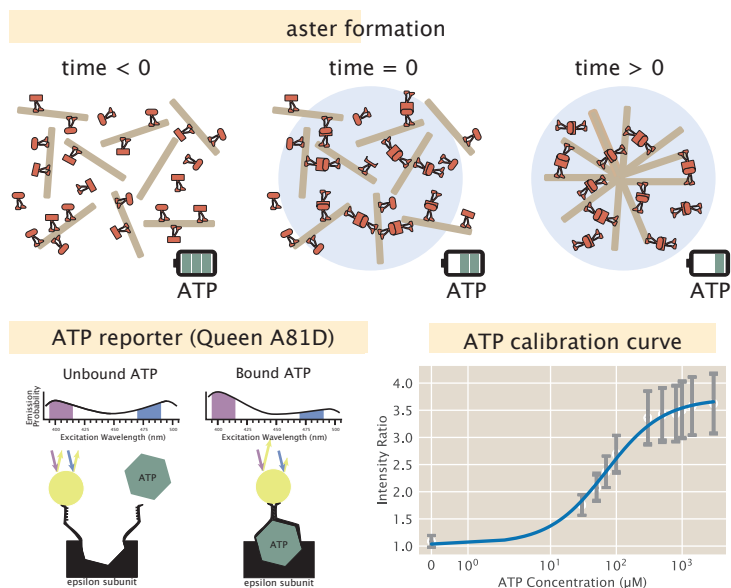


Figure 6.1: Schematic of the experimental system used to measure spatiotemporal evolution of ATP. The top panel illustrates the formation of an aster using light activated motor dimerization. Before light activation, motors independently walk on microtubules hydrolyzing ATP. At $t = 0$ a circular light pattern is projected onto the sample. Motor proteins inside the illuminated region dimerize, crosslinking microtubules. As time elapses, microtubules are dragged into an aster resulting in the depletion of ATP. The lower left panel illustrates the binding mechanism for ATP to the ATP probe. Binding ATP to the probe causes the number of emission counts due to an excitation of 405 nm light to increase, while emission counts from 480 nm light excitation decreases. By comparing the ratio of the emission counts at 405 nm and 480 nm light excitations, the concentration of ATP can be inferred. The lower right panel is a calibration curve mapping known ATP concentrations to fluorescent light intensity ratios. Each black circle represents the mean ratio value for a given image and gray error bars report the standard deviation of the image.

the data was interpreted using quantitative models completely and rigorously transparent.

6.3 ATP Concentrations in Space and Time

The key elements of our experimental design are shown in Figure 6.1. As noted above, using spatially and temporally controlled illumination, we can generate patterns such as the radially symmetric aster shown in the schematic. Our principal experimental goal is to measure the rate of consumption of ATP as a function of position and time, a goal that is realized by using the fluorescent, ratiometric ATP reporter [9] depicted in Figure 6.1(B). The probe mechanism creates a change in the protonation state [10] of the fluorophore when ATP binds [11], triggering a shift in the fluorophore's absorption spectrum [12]. By using known standards, as shown in Figure 6.1(C), we can construct a calibration curve that now permits us to measure the ATP concentration in a given spatial region. Given that the characteristic scale of ATP concentrations in our experiments are of order 100 mM, we see that our ATP probe can measure ATP consumption in a spatially resolved fashion since, as seen in the calibration curve, the ATP reporter is sensitive in precisely the concentration ranges over which the reporter is effective. Note that in the Supplemental Information, we give an extremely detailed description of how we handled uneven illumination and photobleaching, a prerequisite to a calibration curve like that shown in Figure 6.1(C).

With this measurement scheme in hand, we were able to characterize the quantity of ATP in space and time during aster formation, like that shown in Figure 6.2. Note that since we measure the ATP concentration from one time step to the next, this allows us to characterize ATP dynamics, namely ATP movements due to diffusion and depletion of the molecules as they are hydrolyzed by molecular motors. In particular, as shown in Figure 6.2(A), we monitor the amount of ATP as a function of distance from the aster center over a period of

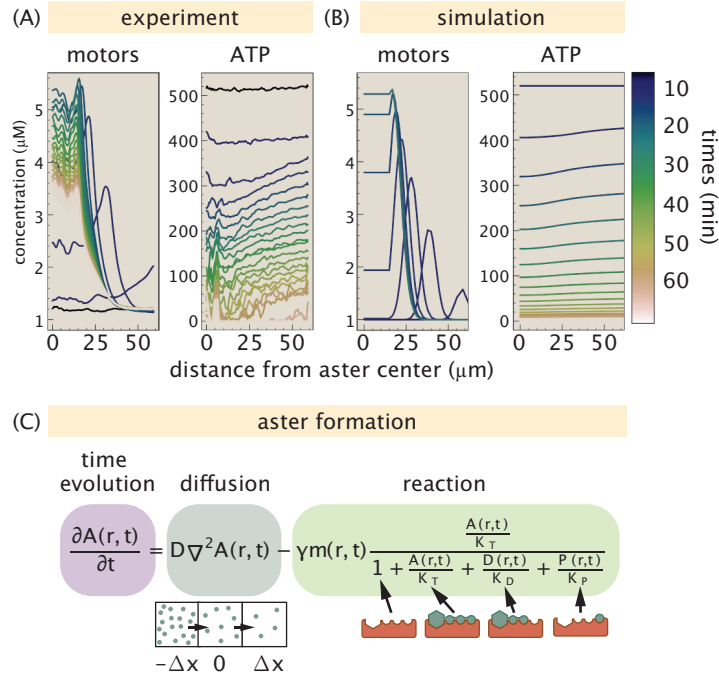


Figure 6.2: Experimental measurements and Finite Element simulations of ATP concentration in space and time. (A) The radial concentration profile of motors and ATP as measured experimentally. (B) The radial concentration profile of motors and ATP as computed using finite element calculations of a continuum reaction-diffusion equation. As time evolves, motor proteins become concentrated near the aster center while ATP depletes. Both the experiment and simulation find emergence of an ATP gradient with the greatest depletion in the aster center, where motors are most concentrated. (C) The reaction-diffusion equation used to simulate ATP concentration is written with illustrations of the diffusive term and the binding states of ATP (A), ADP (D) and phosphate (P) to the motor protein schematized in red.

more than an hour, revealing not only the development of a spatial gradient, but also a net overall consumption of ATP.

Another way of visualizing the results of our measurements is to take the ATP data from successive instants and convert it to a power. As shown in Figure 6.3, the power can be evaluated directly in units of ATP/s, making it possible to compare directly to other measurements, as well as estimates of what the energy from hydrolysis events is used to pay for. Next, we ask how one might develop quantitative intuition for the distribution of the ATP in the aster in both space and time.

The profiles revealed in Figure 6.2 characterize the radial and temporal dependence of

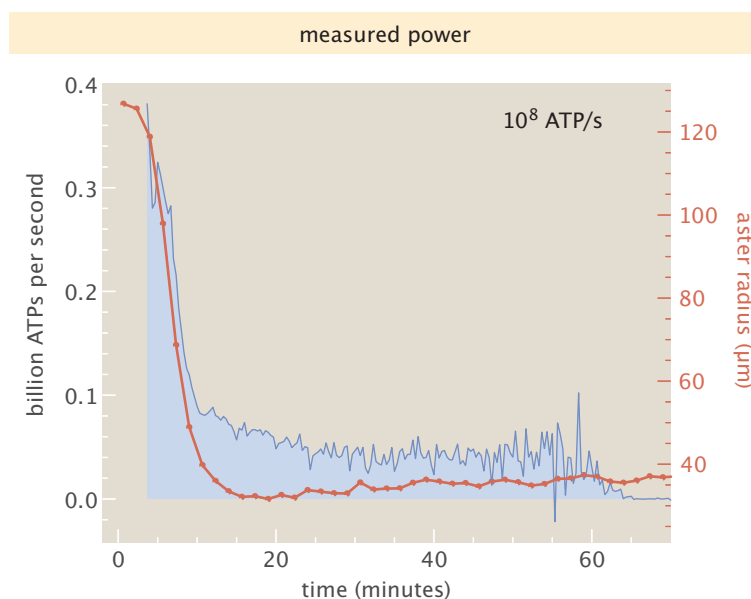


Figure 6.3: The power of aster formation. The number of ATPs consumed per second is plotted as a function of time in light blue. The red line on top reports the aster radius over time. The magnitude of power follows the size of the aster, consuming ATP faster while contracting and reaching a baseline power level once the aster is no longer changing size.

both the motors and the ATP. As seen in the dynamical equation in Figure 6.2(C), the change of concentration in some small material volume element can be attributed both to ATP molecules entering and leaving that small region and to the hydrolysis of those ATP molecules by molecular motors that are in the material volume element of interest. More precisely, as shown in the figure, we can write a reaction-diffusion equation that captures the rate of change of ATP in a material volume element. Note that there is another more pernicious dynamics taking place during our experiments, namely, photobleaching. (We have several sections in Supplemental Information appendices that describe how we measure and account for photobleaching, including finite element simulations of a diffusion-photobleaching equation.) As shown by the box underneath the equation in Figure 6.2(C), a material volume element of interest has molecules both leaving and entering as a result of diffusion, as described in more detail in the Supplement. The reaction term uses a Michaelis-Menten-like dynamics for the rate of ATP consumption by

motors. However, the denominator of that term also includes terms that reflect competitive inhibition of the reaction due to ADP and P_i . The local hydrolysis rate is proportional to the density of motors and to the concentration of ATP itself.

One convenient way to analyze the solutions to such equations in diverse geometries is by appealing to numerical methods. In our case, we used the finite element method to compute the space-time history of the ATP as shown in Figure 6.2(B). As seen in the figure, for reasonable parameter choices (the details of parameter selection are described in the Supplement), the time evolution of the ATP field is in reasonable accord with our measurements. One question that naturally arises is to ask what that ATP hydrolysis “pays for?” Of course, mechanistically, we know that the ATPs are being consumed by motors as they carry out their walk along microtubules. But here we mean it differently. That energy is dissipated through elementary processes such as ordering and contraction of the microtubule network. How much energy do these processes cost?

Microscopically, a huge variety of dissipative processes are taking place during the microtubule-motor rearrangements attending aster formation. As shown in Figure 6.3 the power varies considerably at different stages of the aster formation process. To that end, we explore the power associated with a variety of processes that we imagine are taking place concurrently and would cost different amounts at different stages of the aster formation process. Indeed, as seen in Figure 6.4 there are a number of possible estimates that one can make in units of ATP/s that reflect what ATP hydrolysis might be used to “pay for.” Each of these estimates (and several others) is spelled out in detail in Supplemental Information appendices; here we simply describe the concept of each calculation and how much power consumption it implies.

Given the measured power, we were intrigued to compare that power to the powers associated with several elementary dissipative processes shown in Figure 6.4. For example, as is evident from the radius as a function of time in Figure 6.3, the volume of the

microtubule aster is decreasing over time. As shown in Figure 6.4(A) and described in detail in elsewhere in appendices, we can perform a simple estimate of the power associated with this contraction as the pV work done divided by the elapsed time. We find that the pV power is five orders of magnitude smaller than the measured power. As shown in Figure 6.4(B), a second dissipative process is the frictional sliding of the various microtubules during the contraction process. A naive estimate is obtained by replacing a given microtubule by a corresponding sphere of the same dimensions and to work out the Stokes drag. As in the case of the pV power, this results in a power that is five orders of magnitude smaller than the measured power of Figure 6.3. A better estimate can be made in which the microtubule is treated as a rod rather than a sphere and in this case the computed power is even smaller. Another approach to estimating the power is offered by field theories of nematic ordering in which the state of the system is characterized by the spatially varying tensor $Q_{ij}(\mathbf{f}, t)$. This estimate is trickier to make since the parameters in such a field theory of motor-microtubule systems are not well known. Nevertheless, as seen in Figure 6.4(C) (and described in more detail in the Supplement), the power we estimate associated with such ordering is many orders of magnitude smaller than the measured power. The final dissipative process highlighted in Figure 6.4(D) is that of maintaining a nonequilibrium gradient of motors radially outward from the center of the aster. One way to think about such a gradient is that if there were not some active transport carrying motors towards the aster center, then diffusion would smooth out that gradient. As we show elsewhere in appendices, there is a well defined prescription for estimating the power to maintain such a gradient and we find that it is of the same order of magnitude as the measured powers. This interesting result suggests the hypothesis that a significant fraction of the ATP hydrolysis consumed by the motors is “spent” to maintain this gradient.

It is practically a cliché to note that living organisms are “out of equilibrium.” But we also find that this compact, binary statement, while true, is largely unhelpful. Equilibrium ideas

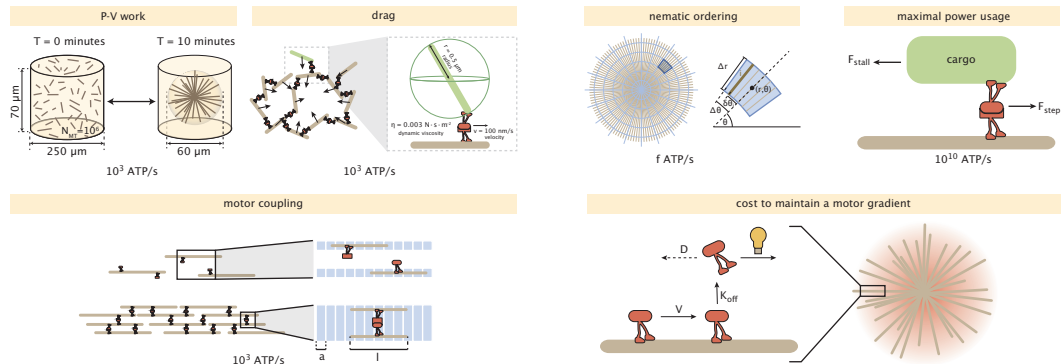


Figure 6.4: Mechanistic processes taking place during aster formation and their estimated power. Each schematic considers a different dissipative processes that occurs during the formation of an aster. (A) Schematic of the power of compressing an “ideal gas” of microtubules. (B) The power of dragging microtubules through a viscous medium. (C) Power estimate for inducing nematic ordering in a random array of microtubules. (D) The power to maintain a concentration gradient of motors in the aster.

are even used to describe the conditions in a star, and it behooves us to have quantitative measures of what we mean by the word “nonequilibrium” in different contexts. For example, although a bacterium can be thought of as a furnace burning fuel at a rate of 10^5 W/m^3 , for single cells and even embryos, this power consumption leads to a temperature change relative to the external world of less than 10^{-7} K . Though each ATP hydrolysis event leads to a local temperature change of $\approx 10 \text{ K}$ in a 1 nm^3 volume, that temperature change relaxes away at time scales far less than a microsecond. Similarly, the concentration gradients of morphogens are very shallow implying that the “out of equilibrium” concentration gradient is barely discernible at the molecular scale. To see this, we imagine discretizing the anterior-posterior axis of the fly embryo into little $1 \mu\text{m}^3$ boxes, the measured gradients are so gentle that two adjacent $1 \mu\text{m}^3$ boxes near the anterior part of the embryo would have an average of 60 and 59 molecules, respectively. The point here is that the question of the extent to which biological systems depart from equilibrium is both important and subtle.

In recent years, renewed efforts have been made both experimentally and theoretically to explore the deep question of when and where energy is being consumed within cells and what the energy consumption is used for. A seminal study in the 1970s by Stouthammer

already recognized the huge energetic burden of protein synthesis in fast growing cells. [13] This process sets a baseline of roughly $10^6 \text{ ATP}/\mu\text{m}^2 \text{ s}$ because to produce the $\approx \text{few} \times 10^2$ peptide bonds per protein each of which costs 4 ATP equivalents for the roughly $\text{few} \times 10^6$ proteins per cell results in a total cost of $\approx \text{few} \times 10^9$ ATPs over the 1000 seconds of the cell cycle. Recent experiments in bacteria illustrated how in starved cells after roughly 24 hours they die due to a loss of membrane potential. Here too, we can make simple estimates of the power required to maintain the transmembrane potential against membrane leakage. Stated simply, the flux of ions out of the cell can be estimated as $dn/dt = JA$, where $J = p\Delta c$. Using the very low permeability to ions of roughly $p \approx 10^{-9} \text{ nm/s}$, we find that the net loss of ions occurs at a rate of 1 - 10 ions/s. Using the relation $\Delta Q = C\Delta V$, we can estimate that in under 24 hours the membrane potential will be nearly completely lost. The power to maintain the membrane potential against such leakage is a tiny value of 10s of ATPs per second.

We argue that the management of energy expenditure in space and time is of critical importance for understanding everything from the development of membrane potentials to the fidelity of biological polymerization to the assembly of structures such as the spindle. Recently, both calorimetry and fluorescence approaches have made it possible to dissect the dissipation in living cells. Such measurements generally tell a similar story, namely, that the scale of measured dissipation is often orders of magnitude larger than the dissipation one might expect by considering elementary processes such as flow. In this paper, we aimed to rigorously measure such energy consumption in space and time in the specific context of a microtubule-motor system.

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