*Chapter 1*

# INTRODUCTION

"What work is worth dedicating your twenties to?" This question, oft posed by my thesis advisor, Rob Phillips, has resonated with me throughout my six years at Caltech. After some introspection into the question of what motivates me, I came to an answer that is perhaps pedestrian: I find the unknown compelling. Naivete which creates a sense of magic and awe, attracts me to problems. And then, an accompanying itchiness tied to unresolved questions summons stubbornness. This internal one-two punch makes it easy to devote my twenties to science. But now, we must ask, what is the most unknown problem to invest in.

Of course, we may not agree here; however, I am hard pressed to find something more mystical and more uncharted than the natural world's ability to sustain life. I am far from the first to share this feeling. I would be remiss here if I did not mention the great Erwin Schrödinger, who delivered a series of lectures entitled "What is life?" where he expressed his own fascination, noting "These facts are easily the most interesting that science has revealed in our day." Despite being a founder of the field of quantum mechanics, even he could not escape wondering "how can the events in space and time which take place within the spatial boundary of a living organism be accounted for by physics and chemistry?" [1]. Regardless of their field of study, many of the great thinkers have been intrigued by questions that surround the ability to distinguish living from non-living systems. I present here a very brief sampling of charming stories, that I do not assert are complete, but rather, that give an impression for how long humans have been wondering about the nature of life.

Beginning with Antony van Leeuwenhoek, this Dutch scientist of the late 1600s used a microscope to explore the world inside a drop of rain water. Here, he found what we know today as microbes. He was entranced to find that these little particles inside water could move and change shape, noting "When these *animalcula* or living Atoms did move, they put forth two little horns, continually moving themselves" [2]. Thus began an adventure searching for the animalcula in water collected from different sources and, comedic to the present day reader, collecting infused pepper water with a pepper, due to van Leeuwenhoek's interest in the " pungency of pepper upon our tongue." Examining the different sources, he began tabulating the differences in size,

shape, quantity, swimming speed, and more. Upon reading his correspondence, it is clear van Leeuwenhoek was entranced by the dynamical nature of the animalcula including their motion and ability to bend their shape, which motivated him to consider these tiny specimens as a new part of the phenomena of life.

Precisely 150 years later, Robert Brown published a study motivated by a very similar spirit, watching the motion of pollen grains[3]. As a botanist, tasked with enumerating as many possible species as he could while traveling, Brown became enamored with collecting and observing pollen under his microscope. The lively movement of the grains inspired Brown's initial thought that the grains were living. However, in continuing the examinations of pollen after the source plant had died, he revised his thoughts stating he "expected to find these molecules in all organic bodies: and accordingly on examining the various animal and vegetable tissues, whether living or dead, they were always found to exist." This finding caused Brown to revaluate his notion of what constituted as living and he started exploring the motion of particulates from inanimate sources like glass. Despite finding that these movements occurred in all forms of tiny matter, Brown was still curious how the motions impacted living systems. He "was desirous of ascertaining whether the mobility of the particles existing in organic bodies was in any degree affected by the application of intense heat to the containing substance." Of course, as we know today, heat, or thermal energy, is precisely the driver of diffusion. Yet, this line of questioning is interesting for two reasons: First, despite Brown's identification as a botanist, he found himself forced to ask physical questions of living systems. Second, his studies, nominally about the role of pollen in plants, touch upon the larger theme of what characteristics define matter as living and what processes facilitate life.

Several decades later, Charles Darwin published his revolutionary work, "On the Origin of Species"[4]. Like Brown, throughout his travels, Darwin explored different species and worked to understand their relations. As we well know, he reached the conclusion "that each species had not been independently created, but had descended, like varieties, from other species." In conceptualizing the notion of lineages, Darwin was left wondering about what properties make different types of life unique. He suggested categorizing species by evaluating "whether any form be sufficiently constant and distinct from other forms, to be capable of definition." This led him to take note of the traits that were preserved among living systems noting, "all living things have much in common, in their chemical composition, their

germinal vesicles, their cellular structure, and their laws of growth and reproduction." These observations ride on the underlying mystery of what constitutes life. Simply reflecting upon the title of this historical work, "On the Origin of Species," we realize Darwin is fundamentally asking where does life begin and how did it diverge from non-living matter?

Moving about another 100 years forward, we return to our discussion of Schrödinger, who delivers his lecture series "What is life?"[1]. Schrödinger ruminates on how living matter is distinct due to its "orderly and lawful behavior of matter, not based exclusively on its tendency to go over from order to disorder, but based partly on existing order that is kept up." This astute sentiment still puzzles us today. Noting his statement that living systems exhibit "admirable regularity and orderliness, unrivaled by anything we meet with in inanimate matter," it feels we must explore the rudimentary cause of this difference by probing the physical parameters we know to be at play in creating orderliness. The amount of order in a system can be described by the system's entropy, which is intimately connected to energy. If perhaps, we could better understand how living systems use and direct their energy, we may be able ascertain hints toward how life is sustained.

In his final chapter, Schrödinger professes "We must therefore not be discouraged by the difficulty of interpreting life by the ordinary laws of physics... we must be prepared to find a new type of physical law prevailing in it." Today, there are many great works aimed to better understand life through the exploration of energy. Here, I provide a smattering of studies that I, once again, do not profess to be complete, but that I find highly inspiring in answering Schrödinger's call to action.

One category of studies includes recent work that investigates a biological system's energy expenditure through calorimetry [5]. This technique measures the power, or energy per time, via temperature sensing in a fluid sample. Exciting work from 2019 quantified the heat output of zebrafish embryos using this method. Amazingly, the result directly informs us how much power these cells are using at a given time. While impressive in its own right, this work additionally explores the cost of mitotic cell cycles. On top of the basal energy level, the authors find oscillations in the power output of their samples. They suspected that these oscillations may be connected to the cell cycle. By adding pharmacological inhibitors, they test how perturbing cell cycle oscillations modifies the measured power oscillations, and indeed find the oscillations disappear. This work, probing both energetics at the cellular level and the individual cellular processes level, takes us a large step closer to understanding

how the basic unit of life, cells, rely on energy.

Subsequent work aims to continue probing the energetic consumption of individual cellular processes. This is very useful to the scientific community as we can begin to create a cellular budget, mapping how much energy cells use for any given function. Determining the energy partitioned to an individual process can be challenging, requiring high enough resolution to measure small heat outputs from small samples. Scientists designed and built a picocalorimeter that reports power outputs at picowatt sensitivities with microliter volumes [6]. With this tool in hand, researchers measured the thermal dissipation of an *in vitro*, active nematic fluid, containing motor proteins and microtubules, which are critical to cell division, transport, and cellular structure [7]. Through this work, they discovered $10^9$ times more energy is dissipated than they predicted based on estimating the power of viscous flows. This work echoes Schrödinger's sentiment that living systems do not always play by the standard physical laws. These systems are maintained in a nonequilibrium steady state, defying the state of maximum entropy demanded in equilibrium. From this result we see how probing the physical laws that we accept as a community can help us interpret living systems and dictate where life seems to harness physics a little differently.

Simultaneously, others have investigated cellular energetics through the study of metabolism. These works hinge upon knowledge of the chemical pathways that intake oxygen and carbon sources, intermediately produce ATP, and output heat and byproducts like NADH. Upon measuring molecules inputted or outputted in metabolism, one can infer the associated energy flux based upon stoichiometry. A 2021 review paper does a beautiful job highlighting works featuring metabolic based techniques [8]. A central theme to this review is the value of measuring gradients in addition to global measurements. Using respirometers to quantify oxygen concentration or calorimeters to measure heat both describe energy fluxes across the entire system, what we refer to as a global measurement. When asking questions involving individual cellular processes, measurements that offer spatial knowledge can be useful in understanding where energy is localized. Some of the same authors wrote a paper developing a technique to image NADH molecules across mouse oocytes [9]. Using fluorescence lifetime imaging, this study explores the emergence of NADH gradients upon imposing various metabolic stresses to oocytes. This breakthrough result opens the door for future work to estimate ATP gradient formation across the oocyte, leading toward an understanding of where

cells allocate energy.

Inspired by the spirit of these great thinkers, both historical and modern, I hope to join the peloton of those using physical approaches to explore the most fundamental of biological questions: what does it mean to be alive? In the thesis that follows, I describe three interconnected stories. These threads strive to paint a unified picture of the energetic and mechanical assembly of motor proteins and microtubules into structures that resemble mitotic spindles, the complex molecular machines that segregate chromosomes during cell division. In the work described, we introduce a new method for direct measurement of ATP molecules in space and time, building upon the field's excitement towards witnessing gradients in isolated processes. We additionally write mathematical models exploring the physics of building and maintaining gradients in non-equilibrium steady states. And, in the spirit of comprehensively understanding our system, we explore the material properties of dynamic network formation.

All of the work described here uses an *in vitro* motor-microtubule model system. It has been well established that combining motor proteins and microtubules with ATP, spontaneous network formation will occur [10, 11]. Tuning the concentration ratios of motors to microtubules, two different structural regimes are observed [12]. The first is termed a nematic regime. Here, microtubules align in very long bundles resulting in swirly, filamentous patterns. A second type of network occurs in what is called a polar regime. Here, radially symmetric star-shaped structures are formed, which are called asters. The poles of the mitotic spindle are in this regime. All the work described in this thesis is in the polar, aster regime. Previous research in our lab aimed to control when and where we create structures. To achieve this goal, a 2019 study from the Matt Thomson lab in collaboration with our group engineered motor proteins to have an optogenetic protein linker attached on their tail [13]. When illuminating the linker, it undergoes a conformational change that, like a puzzle piece, allows it to attach to a linker on another motor's tail [14]. With this system, we can project any pattern of light onto a sample and initiate structure formation in the illuminated regions.

In Chapters 2 and 3, we measure spatiotemporal gradients of ATP molecules in dynamically forming asters. We introduce a fluorescent-based ATP reporter into our experimental system, which binds and releases ATP without hydrolysis. Upon excitation, the fluorescent signal of the probe changes based on the binding state of ATP, providing a direct readout of ATP gradients across an aster. With this measure-

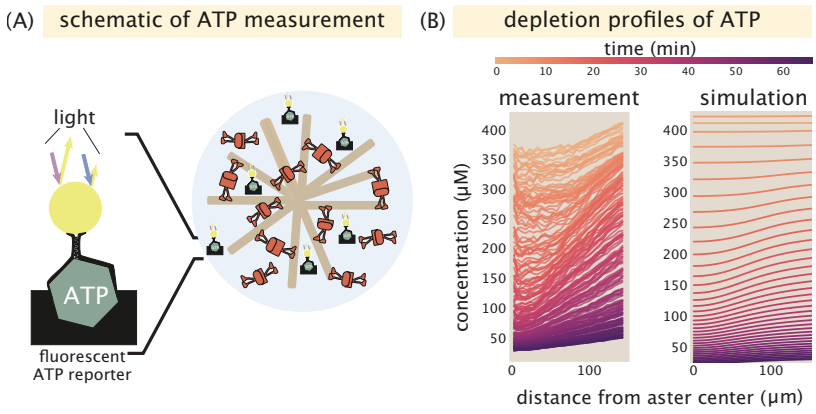


Figure 1.1: **Measuring ATP in space and time.** (A) Cartoon model of the ATP measurement scheme. (B) Experimental and simulated results showing emergent ATP gradients along the aster radius.

ment in hand, we work to interpret the cost of aster formation with reaction-diffusion modeling, finite element simulations, and systematic estimations of dissipative processes. A summary of this project is depicted in Figure 1.1.

Motivated to understand the gradients we observe in the previous chapters, in Chapter 4, we use statistical physics to develop a theory of biological gradients. We write flux-based equations that compare the molecular kinetics due to diffusion versus active transport. This allows us to predict the energy paid to simply maintain gradients as well as the additional costs required to steepen gradients. Through the mathematical description we provide, we aim to shed insight into the energetic expense of creating and sustaining gradients, allowing living systems to exist in non-equilibrium steady states. A preview of the story to come is depicted in Figure 1.2.

A comprehensive understanding of gradient formation in our system requires knowledge of the aster's material properties. Chapters 5 and 6 describe a project that used photobleaching techniques to observe advection and diffusive spreading during active network contraction. This study investigates which mechanism dominates upon tuning motor speeds by varying motor species and ATP concentrations. Regardless of motor speed, we find the role of advection is greater than diffusion. However, interestingly, we find that the rate of diffusion scales with motor speeds, indicating diffusion in this contracting network is an active process. Exploring deformations of contracting regions in the aster provide a material based perspective from which we build our understanding of the formation of gradients. In Figure 1.3 we provide

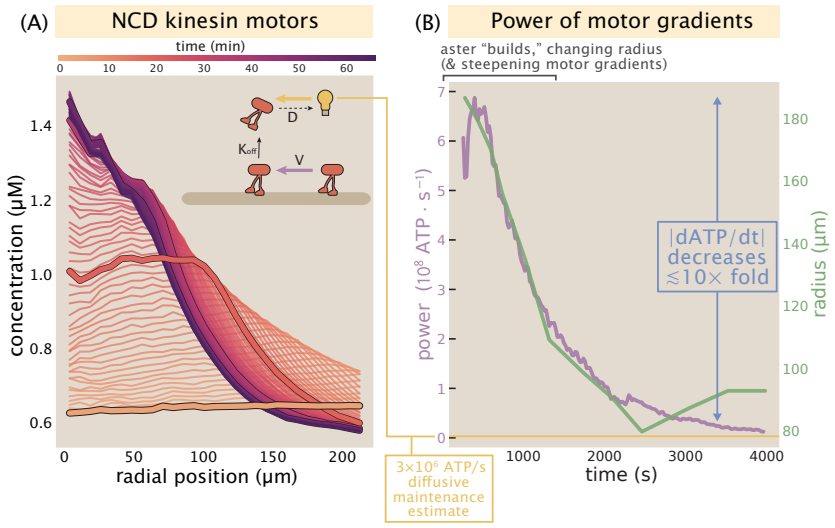


Figure 1.2: **Motor gradients emerge at the expense of ATP dissipation.** (A) Measurements of motor proteins in a developing aster. The cartoon inset highlights the competition between advection toward the center of the structure versus diffusion. (B) Measurements of the power dissipated from the building and maintenance of an aster. We note upon forming a steady-state gradient, the power expenditure drops nearly an order of magnitude.

a graphical overview of this work.

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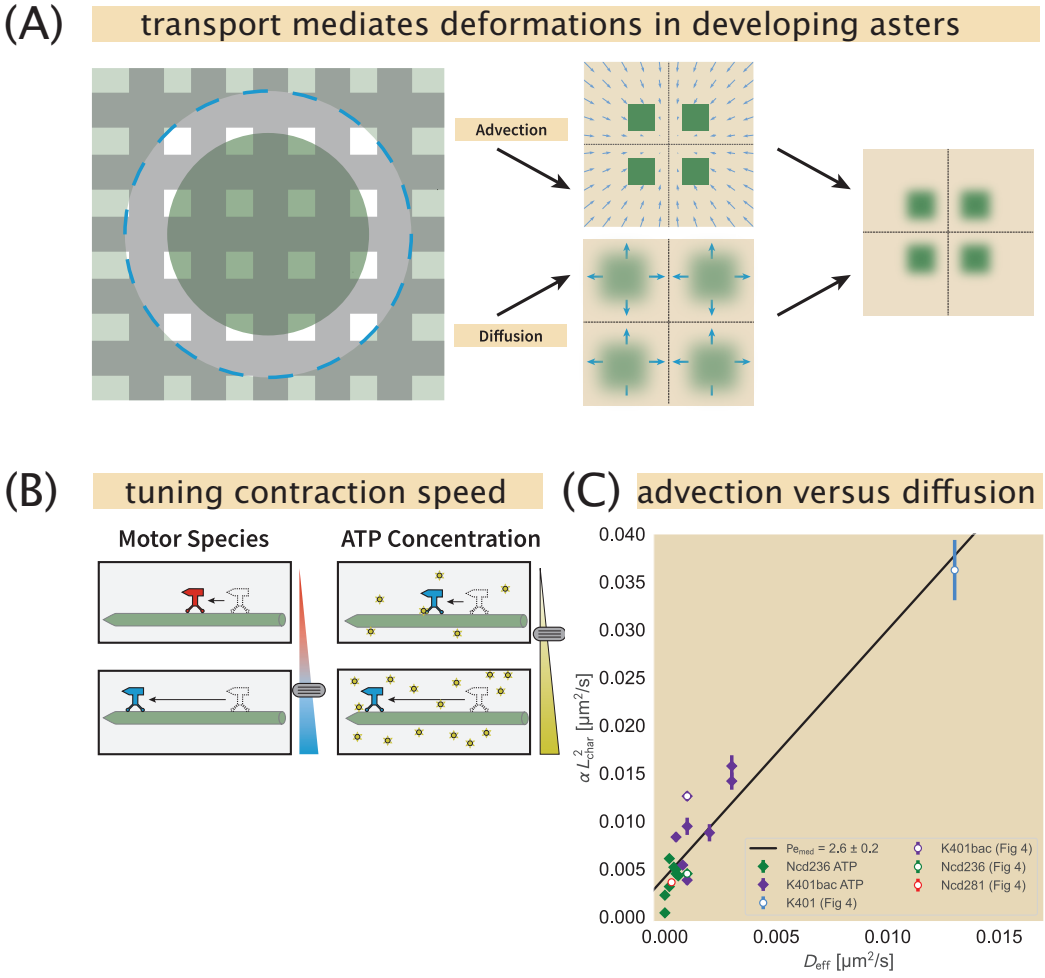


Figure 1.3: **Photobleaching grid patterns allow visual and quantitative comparisons of the dominant transport mechanisms.** (A) Cartoon scheme of the photobleaching system. Tracking unit cell deformations allow an analysis of the roles of advection versus diffusion. (B) Representation of the parameters that modulate motor speed. (C) The magnitude of advection and diffusion scale linearly with each other.

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