UNIVERSITY OF CALIFORNIA SAN DIEGO

Meditations on Molecular Motors

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by

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This dissertation of Andrew Bollhagen is approved, and is acceptable in quality and form for publication on microfilm and electronically.

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True story. I had just seen the Disney musical Moana. Wait, who am I kidding. I had just watched the Disney musical Moana for probably the tenth or eleventh time. Whatever the exact number, it was sufficient for its soundtrack to have become the soundtrack of my inner life when I arrived at UC San Diego for the Philosophy Department's prospective student's visit week. Our group was standing at the Torrey Pines Gliderport, overlooking the ocean. A lyric rang through my head. *There's a line where the sky meets the sea and it calls meeee, and no one knooooows how far I'll gooooo*. Might this be a bit corny? Absolutely. Am I ashamed to say that I was, in that moment, very much feeling like a Disney Princess at the threshold of a great adventure? No. Well, maybe a little embarrassed . . .

The lyric was true though. Nobody, me included, knew how far I'd go. In retrospect, though, one thing is certain. I wouldn't have gotten anywhere without the support of a great many people the first among them being my wife, Grace, to whom, *as the wind in my sail on the sea stays behiiind meeeee,* I dedicate this dissertation. Grace, I can't even begin to thank you enough for your support, and sacrifice. I love you.

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Chapter 3, in full, is a reprint of the material as it appears in Bechtel, W., & Bollhagen, A. (2021). Active biological mechanisms: Transforming energy into motion in molecular motors. *Synthese*, *199*(5), 12705-12729. Chapter 4, in full, is a reprint of the material as it appears in Bollhagen, A., & Bechtel, W. (2022). Discovering autoinhibition as a design principle for the control of biological mechanisms. *Studies in History and Philosophy of Science*, *95*, 145-

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ABSTRACT OF THE DISSERTATION

Meditations on Molecular Motors

by

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Doctor of Philosophy in Philosophy

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My dissertation represents the first sustained philosophical treatment of the science of *molecular motor proteins*—proteins that transform the chemical energy stored in ATP into mechanical motion. The analysis proceeds from a broadly mechanistic philosophical perspective, albeit one that has witnessed two recent developments.

First, philosophers both within and in the orbit of mechanist philosophy of science have recently turned philosophical attention to how scientists *characterize* phenomena, as opposed to explain them, the latter being New Mechanism's traditional focus. The first section, *Characterization* (Chapters 1 & 2), contributes to this still nascent philosophical discussion. I

draw case studies from the history of cell biological research on motor proteins to analyze key experimental practices by which scientists succeeded in *characterizing* (as opposed to explaining) the activity of molecular motor proteins both quantitatively (Chapter 1) and qualitatively (Chapter 2).

Second, the perspective on biological mechanisms that I adopt is a "revisionist" one initially formulated by my supervisor, William Bechtel, and his former graduate student Jason Winning, that construes biological mechanisms as *sets of constraints on the flow of free energy.* Each chapter of the second section, *Explanation* (Chapters 3, 4, &5), extends this philosophical view in connection with analyses of the explanatory practices of molecular motors researchers.

Meditations on Molecular Motors

Preface

This dissertation represents the first sustained philosophical treatment of the science of *molecular motor proteins*—proteins that transform the chemical energy stored in ATP into mechanical motion. The analysis proceeds from a broadly mechanistic philosophical perspective, albeit one that has witnessed two recent developments. First, as reflected in the first two chapters, philosophers both within and in the orbit of mechanist philosophy of science have turned philosophical attention to how scientists *characterize* phenomena, as opposed to explain them, the latter being New Mechanism's traditional focus. Second, as reflected in the third, fourth, and fifth chapters, the perspective on biological mechanisms that I adopt is a "revisionist" one initially formulated by my supervisor, William Bechtel, and his former graduate student Jason Winning that construes biological mechanisms as *sets of constraints on the flow of free energy* (Winning and Bechtel 2018).

Each of the dissertation's two sections represent contributions to these two recent developments. Part 1 is entitled ***Characterization*** (Chapters 1 and 2) and Part II, ***Explanation*** (Chapters 3, 5, and 5). Each chapter of ***Characterization*** discusses episodes drawn from the history of cell biological research on molecular motors proteins in which researchers developed and deployed novel experimental techniques to characterize (as opposed to explain) the movement of molecular motors both quantitatively (Chapter 1) and qualitatively (Chapter 2). Each chapter of ***Explanation*** analyzes the explanatory strategies researchers deployed in developing mechanistic accounts of the means by which motor proteins move in the characteristic ways they do. I discuss each chapter in turn.

Chapter 1, entitled **Modeling and Measuring: *In Vitro Reconstitution* in Philosophical Perspective,** presents two case studies in which scientists developed *in vitro* reconstituted

systems (IVRSs) to study the molecular motor protein myosin and its "partner protein," actin. My analysis situates both case studies in the philosophical literature on modeling. The first case study involves researchers developing an *explanatory* IVRS to identify the molecular mechanism that drives muscle contraction. On my analysis, explanatory IVRSs are "common features models" that are built to "exemplify" (i.e., instantiate and refer to) their modeling targets with respect to both the *explanans* and *explanandum*. I discuss the epistemic constraints that such a model must satisfy in order to succeed in its explanatory goals. However, as the second case study shows, *in vitro* reconstituted systems can be developed for purposes other than that of explaining phenomena. In particular, they can be developed as procedures to *characterize phenomenon quantitatively*. That is, they can be developed for the purposes of *measurement*. My analysis contrasts the epistemic constraints that a measurement IVRS must satisfy with those that an explanatory IVRS must satisfy. In contrast with an explanatory IVRS which must exemplify some particular target phenomenon of interest (e.g., muscle contraction, protein synthesis etc.), a measurement IVRS must exemplify the quantity to be measured. I illustrate the practice of building a measurement IVRS through a case study in which researchers developed such a system to measure the characteristic rate at which myosin moves over actin.

Chapter 2, entitled ***The Inchworm Episode: Reconstituting the Phenomenon of Kinesin Motility*** analyzes a case in which researchers studying the motor protein *kinesin* used an *in vitro* reconstituted system called the *single molecule motility assay* to characterize the manner in which the motor protein *kinesin* "walks" along its cytoskeletal "track." In contrast to the *measurement* IVRS discussed in the previous chapter, the researchers discussed in Chapter 2 developed and deployed their reconstituted system to characterize the phenomenon of molecular motor movement *qualitatively*. Additionally, as I argue on the basis of the case study discussed in

this chapter, what mechanist philosophers of science have called *phenomenon reconstitution*—scientists coming to substantively re-characterize the target of their investigation—can occur even in the context of experimental research aimed at characterizing, as opposed to explaining, phenomena. This departs from extant philosophical analyses of phenomenon reconstitution which have analyzed it as driven by researchers gaining explanatory insight into the phenomenon in question.

The dissertation's second section, ***Explanation***, begins with **Chapter 3**: ***Active Biological Mechanisms: Transforming Energy into Motion in Molecular Motors*** (published as Bechtel & Bollhagen 2021). This chapter represents an extension of the "revisionist" philosophical understanding of biological mechanisms that I alluded to above. Formulated initially by William Bechtel and his student Jason Winning, I take Winning's baton and apply, with Bechtel, the "revisionist" framework to offer an analysis of molecular motors researchers' mechanistic accounts of how molecular motor proteins generate motion. On the account we develop, molecular motor proteins—indeed, biological mechanisms in general—are to be analyzed as *sets of constraints on the flow of free energy.* In addition to offering an analysis of the explanations these biologists give, **Chapter 3** draws out the implications of this view for New Mechanism's traditional analytic categories of "entities" and "activities." Traditionally viewed as fundamental ontological categories of mechanist philosophy, they are typically regarded as not subject to further analysis or explanation. On the view we develop in the chapter, however, "entities" are analyzed as "constraints" on the flow of free energy which enables us to *explain "*activities" as the flow of free energy through constraints.

Chapter 4 entitled ***Discovering Autoinhibition as a Design Principle for the Control of Biological Mechanisms*** (published as Bollhagen & Bechtel 2022) describes how molecular

motors scientists discovered that motor proteins *kinesin* and *dynein* "autoinhibit," i.e., adopt a functionally distinct conformation in which they do not generate movement or hydrolyze ATP. With the help of this historical case, the chapter shows a central tenant of traditional mechanist philosophy—that phenomena are individuated in terms of the single phenomenon they explain or the single function they perform—is false. The "revisionist" conception of biological mechanisms, however, can accommodate the fact that biological mechanisms can produce more than one phenomenon depending upon how they are *controlled*. Control processes regulate the behavior of mechanisms by altering the constraints characteristic of the controlled mechanism. As the case studies illustrate, under one regime of control, kinesin and dynein produce motility. Under another regime of control, those same mechanisms autoinhibit. Additionally, **Chapter 4** argues that the mechanist tradition's analysis of mechanism *discovery* on which the process proceeds in a "phenomenon-first" way, should acknowledge that it can also proceed "mechanism-first." That is, rather than thinking that the process of discovery always begins with characterizing a phenomenon to be explained and then identifying the mechanism responsible for that phenomenon, the chapter argues that researchers can attribute to a mechanism already identified as responsible for some phenomenon, responsibility for an additional phenomenon as well.

Chapter 5: ***Process or Mechanism: Implications of Brownian Ratchet Accounts of Molecular Motor Activity*** deploys this "revisionist" account of biological mechanisms in the context of the philosophical debate between mechanists and philosophers who advocate for a "processual" framing of biology (Dupré and Nicholson, 2018; Nicholson, 2020). Nicholson in particular has argued that "Brownian ratchet" explanations for molecular motor movement support a processual approach. On the contrary, this chapter argues that such explanations are in

fact more appropriately analyzed in terms of the "revisionist" mechanist view. Even on Brownian ratchet accounts of molecular motor activity, a source of free energy is required and that energy must be constrained in order to perform work.

In sum, this dissertation represents an initial philosophical analysis of the science of molecular motor proteins from the point of view of an updated mechanist philosophy of science. It also represents a contribution to the development of this updated mechanist philosophical view itself. My hope for the dissertation is that it shows philosophers that the science of molecular motor proteins constitutes a rich vein to mine and that it will inspire others to pick up their philosophical pickaxes and mine it along with me.

References

Bechtel, W., & Bollhagen, A. (2021). Active biological mechanisms: Transforming energy into motion in molecular motors. *Synthese*, *199*(5), 12705-12729.

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Part 1: Characterization

Chapter 1: Modeling and Measuring: *In Vitro Reconstitution* in Philosophical Perspective

Introduction

Building *in vitro* reconstituted systems (henceforth IVRSs) is, according to biologists Gavin Schlissel and Pulin Li, an "experimental strategy that seeks to recapitulate biological events outside their natural context using a reduced number of parts" (Schlissel *et al.,* 2020). The practice involves extracting elements from a living system thought to be responsible for some phenomenon of interest, placing them in an artificial preparation, and attempting to produce the phenomenon "in glass," hence, *in vitro*. Sometimes referred to colloquially as "constructing X in a test tube," where X is some biological phenomenon, this practice is quite commonplace in cell biology. Researchers have succeeded in reconstituting a wide range of cellular phenomena including muscle contraction (which I discuss at length), the motile systems of bacteria, spindle formation during mitosis, and DNA synthesis, just to name a few (Liu & Fletcher, 2009; Rall 2018). Though it is an important experimental practice, little philosophical work has been done to analyze it. The purpose of this chapter is to offer such an analysis.

Section 1 briefly situates my analysis of IVRSs in the context of the broader literature on models in science. I point out that IVRSs are "physical common-features models" that can serve purposes of explanation.[1] A common way of explicating the sense in which models "share common features" with their targets is in terms of *exemplification*. Some philosophers, most notably for my purposes Frigg and Nguyen (2016), analyze exemplification in terms of two further concepts, namely, instantiation and reference. For F&N, a feature of a model (X) exemplifies a feature of the target (Y) just in case X instantiates and refers to Y. I apply the

[1] They can serve purposes of prediction as well. I do not think there anything particularly unique or interesting to be said about how IVRSs can facilitate prediction. My discussion focuses on IVRSs as used for explanation and measurement as it is in the difference between these uses where I think philosophical interest lies.

notion of exemplification to the interpretation of the case study of building an explanatory IVRS for muscle contraction that I present in **Section 2.** I interpret this case in terms of an account of exemplification on which features of an IVRS must "literally" instantiate features of the model's target. As we will see, this account is a restricted version of the notion of exemplification Frigg and Nguyen construct.

Also, in Section II, I follow Nersessian's (2022) focus on the iterative practice by means of which IVRSs are constructed. As she points out, the original notion of exemplification that Goodman and Elgin introduced into the philosophy of science and that Frigg and Nguyen take up "addresses finished representations" (48). Nersessian takes "exemplification to be a dynamic process in which "*models are built toward exemplifying features of a biological system* . . . (48, original italics). Taking cues from Nersessian, by "exemplification" I refer to the concrete process by means of which IVRSs (and other models) are constructed, as well as to the abstract representational relation between model and target. Thus, I am intentionally ambiguous in my use of the term "exemplification." As the case study presented in this section shows, the iterative practice of exemplification (the one illustrated in the case study) represents the means by which researchers pursue their aim to construct a model that stands in the abstract relation of exemplification (the one analyzed in terms of reference and instantiation).

Section 3 presents a second case study which shows that IVRSs can be constructed not just for purposes of explaining biological phenomena but for purposes of characterizing them quantitatively. That is, IVRS can represent physical common-features models that are developed and deployed for purposes of *measurement.* In this case, the iterative practice of building an IVRS that exemplifies the measured quantity is distinct from that by which researchers build an IVRS to explain a particular biological phenomenon of interest. In this practice, researchers

assume, perhaps wrongly, at the outset that there is some characteristic quantity to be measured. For example, as we will see in this section's case study, in building an IVRS to measure the rate at which the motor protein *myosin* moves over its partner protein *actin*, researchers assumed at the outset that myosin moves over actin at some characteristic rate. In fact, as Chang (2009) would point out, they had to on pain of unintelligibility. I make this point in terms of Chang's notion of epistemic activity/ontological principle pairs. In assuming that myosin moves at some characteristic rate, researchers assumed that the rate at which myosin moves has some single measurable value. This is tantamount to, as Chang puts it, "conditionally committing" to the ontological "principle of single value." As I discuss, it is only on the assumption that myosin moves over actin at some characteristic single rate that the epistemic activity of trying to measure that rate is intelligible. My **Conclusion** briefly summarizes the chapter.

Section 1

IVRSs are "physical common-features" models. Physical models are familiar to philosophers; examples include Watson and Crick's metal model of DNA (Schaffner 1969), Phillips and Newlyn's hydraulic model of an economy (Morgan and Boumans 2004), the US Army Corps of Engineers' model of the San Francisco Bay (Weisberg 2013), and Kendrew's plasticine model of myoglobin (Frigg and Nguyen 2016). However, as physical models, IVRSs are distinctive in that unlike, say, Watson and Crick's *metal* model of DNA, IVRSs are constructed out of *biological* material. In fact, in the cell biological practice, the biological material out of which IVRSs are built is supposed to be the same material out of which the modelled system itself is composed.

Common-features models are also philosophically familiar. As Batterman and Rice (2014) characterize them, common-features models are usually *explanatory* in their epistemic purport and derive their explanatory power from the fact that there obtains between the model and the modelled system "some kind of accurate mirroring, or mapping, or representation relation between model and target" (351). Insofar as IVRSs serve explanatory purposes, they do so as common-features models. Indeed, as the example drawn from the history of experimental research on muscle contraction presented in the next section illustrates, the practice of building such explanatory IVRSs is an iterative one in which researchers note that their present iteration fails to "mirror" the modelled system either with respect to the mechanistic *explanans* or the phenomenal *explanandum*. In this way, sharing common-features operates as a constraint on the successful development of an explanatory IVRS.

That said, IVRSs can serve purposes other than explanation. As Nersessian (2022) writes, developing and deploying IVRSs "is an epistemic activity that forms the basis for understanding, explanation and prediction . . ." (52). IVRSs can be used "to *predict* what is going to happen in a system in vivo . . .like people use mathematical models to predict . . . what would happen in real life" (52). While IVRSs can serve as models for prediction, there is a contrast to be drawn between mathematical models and IVRSs that the above quote glosses over, namely, that sharing common features with the modelled system is not a constraint that a mathematical model needs to satisfy in order for predictions made on its basis to be justified. As Milton Friedman claimed, one might be able to predict the behavior of a leaf on a tree using a mathematical model built on the false characterization of the leaf as a rational actor, attributing to it the ability to "deliberately [seek] to maximize the amount of sunlight it receives, given the position of its neighbors, as if it knew the physical laws determining the amount of sunlight that would be received in various

positions and could move rapidly or instantaneously from any one position to any other desired and unoccupied position (Friedman, 191)." While IVRSs can be used, like mathematical models, as a basis for predicting the behavior of the modeled system, this is because they do indeed share common features with the modeled system. As this also grounds their ability to explain, IVRSs pull double explanatory and predictive duty.

Also, with respect to prediction, another contrast to consider is that between IVRSs and other physical models that *do not* literally share features with their modelling target—are not common-features models—but can be used nonetheless for purposes of prediction. For example, Frigg and Nyguen (2017) discuss a scale physical model of the ocean-liner SS *Monterrey* constructed by the Matson Navigation Company that was built to predict the resistance the ship would experience as it moved through water. While the model was carefully constructed to have the same shape as the ship, it was considerably smaller. As a result, the resistance experienced by the model and that experienced by the ship is not a feature they share and, therefore, one to which no bald appeal can be made in justifying a prediction about the resistance the ship will experience on the basis of the resistance the model does. Nonetheless, as I discuss in more detail later, according to Frigg and Nyguen's (2017) DEKI account, modelers can make predictions about the behavior of a modelling target as long as there is a "key" (the "K" in DEKI) that enables the translation of features of the model into features of the modelled system.[2] What particular "key" is used will vary across models. In the case of the ship, the "translation procedure . . . is informed by our theoretical background knowledge about fluid mechanics, and clever ways of thinking about things like scale, length, and resistance" (1). A different theoretical

[2] The relationship between the DEKI account of model *representation* and how, on DEKI, model users are *justified* in the predictions they make using the model is an interesting topic of philosophical discussion. See Millson, J., & Risjord, M. (2022) and Frigg and Nguyen's reply in Frigg, R., & Nguyen, J. (2022). I do not think this issue affects my analysis and so I leave it aside.

background would be invoked if we were justifying a prediction about, say, the impact of a hike in interest rates based on a model of the economy.

To flesh this out in more detail, Frigg and Nyguen's DEKI account offers an analysis of what it is for a model to "share common features" with its target in terms of *exemplification*, a term introduced into the philosophy of science by Goodman and Elgin (Goodman, 1976; Elgin, 2000). The acronym DEKI stands for Denotation, Exemplification, Keying, and Imputation. As Salis et. al.,(2020) state, "Denotation is a dyadic relation that obtains between certain symbols and certain objects" (197). On DEKI, this is the relation that obtains between a model and its target—the model denotes its target. Exemplification is a relation that holds between features of the model and features of the target. Frigg and Nguyen follow Goodman and Elgin in analyzing exemplification in terms of two further concepts—instantiation and reference: "An item exemplifies a property if it at once instantiates the property and refers to it. 'Exemplification is possession plus reference. To have without symbolising is merely to possess, while to symbolise without having is to refer in some other way than by exemplifying.' (Goodman 1976, pg. 53). An item that exemplifies a property is an "exemplar." Put formally, exemplification is defined as follows. "X exemplifies Y if and only if X instantiates Y and refers to Y." Insofar as some model feature exemplifies—i.e., both instantiates and refers to—a feature of the model's target, it is an *exemplar* of that feature. I shall explain what both these are supposed to mean below. A Key ("K" in DEKI) is a scheme of translation that enables model users to translate descriptions of a feature in the model into descriptions that are putatively appropriate to Impute ("I" in DEKI) to the corresponding feature of the target. Imputation is the act of ascribing the "keyed up" feature to the target system.

Frigg and Nguyen's account is general enough to capture simple cases, like a paint chip, as well as more complicated ones like the hydraulic machine consisting of water pipes, reservoirs, levers, and pump that economists Phillips and Newlyn used as a model of an economy (60). Applied to a paint chip like you'd find in the aisle of a home improvement store, the paint chip denotes blue. It also exemplifies the feature blue by instantiating it and, in the context of the practice of using paint chips, referring to it. The analysis is just this straightforward because, as Frigg and Nguyen (2017) state, the paint chip "literally" instantiates blue. The situation is more complicated when we shift focus to Phillips and Newlyn's hydraulic machine. As they write, "A hydraulic system instantiates hydraulic features like having a flow of two liters per minute through a certain pipe; it doesn't instantiate economic features like two million of the model-current being received by the treasury." This might seem to pose a problem. If exemplification requires instantiation, and the features of the hydraulic model to not instantiate the features of the modeling target, then the model does not exemplify and, therefore, does not represent the target. For Frigg and Nyguen, however, "there's an easy fix. Nothing in what we want to do with models depends on features being instantiated *literally* . . .Nothing in our notion of exemplification depends on features being instantiated in a metaphysically robust sense." (60. Original italics).

To explain, they appeal to their notion of an "interpretation." For Frigg and Nguyen, an interpretation is required to turn what would otherwise be just a mundane object into a model. For many objects they

> look nothing like the things they are models for, and, more generally, there is nothing in their intrinsic features that would make them models let alone models for something in particular . . .The fact that some objects are models must therefore be rooted in something other than the idea that they somehow look like the objects that they are models for. We submit that this something else is an *interpretation.* We turn a hydraulic machine into a model for an economy by interpreting some of

> its features in terms of economic features; we interpret the flow of water as the flow of money . . . " (55).

Frigg and Nguyen offer the following technical definition of an interpretation.

> Let X be the object that serves as a model . . . and let X = $\{X_1, \ldots . X_n\}$ be a set of features pertaining to X. Next let Z be the domain we are interested in . . . and let Z = $\{Z_1, \ldots . Z_n\}$ be a set of features pertaining to Z. An interpretation I is a one-to-one mapping from X to Z (56).

This interpretation secures the reference relation between model features and features of the model's target. This is unproblematic. Any feature of any object can, under an interpretation that maps it to a feature of some other thing, refer to that other feature. Instantiation, however, is trickier business. In order to accommodate their analysis of exemplification to models whose features do not literally instantiate the features of their targets, Frigg and Nguyen introduce the notion of *instantiation-under interpretation* or, as they abbreviate it, *I-instantiation*. "The interpretation correlates *X*-features with *Z*-features, and so we can say that the model *I*-instantiates a certain *Z*-feature iff it instantiates the corresponding *X*-features . . . This allows us to introduce the notion of *I*-exemplification, which is exactly like exemplification except that features are *I*-instantiated rather than instantiated" (60 original italics). In this way, they can say that a model whose features do not literally instantiate features of the model can nonetheless *I*-exemplify the target.

I take no issue with Frigg and Nguyen's claim that, for certain models, it is unnecessary for features of a model to literally instantiate features of the target system. However, I do take issue with their claim that "Nothing in what we want to do with models depends on features being instantiated *literally*." As we will see in more detail in the next section, for *explanatory* IVRSs, this is precisely the case.

Section 2

As I mentioned in my introduction, my analysis deploys two senses of the term "exemplification." On the one hand, I use the term to refer to the abstract relation between a model and a target system—i.e., that relationship that Frigg and Nguyen analyze in terms of instantiation and reference. For reasons that the case study will make clear, the abstract notion of exemplification relevant to philosophically analyzing explanatory IVRSs is one on which the model *literally* instantiates the target system. On the other hand, I use the term to refer to the concrete practice of developing an IVRS that stands in the abstract relation of exemplification. In this sense, "exemplification" refers not to an abstract relation to be analyzed in terms of further concepts (e.g. reference and instantiation), but to a concrete practice wherein research draw comparisons between iterations of their IVRS model and the target system as studied using various different tools and techniques. Thus, "exemplification" (in the concrete sense) refers to the means by which researchers achieve a model that "exemplifies" (in the abstract sense) their target. To illustrate the concrete practice of exemplification in detail, I turn now to the case study.

In the mid-nineteenth century, Wilhelm Kühne isolated what he took to be a single viscous molecule from muscle-press juice that he called *myosin* (Kühne 1864). Later, experimenting with Kühne's isolate in the wake of the discovery of adenosine triphosphate (ATP) and its identification as a biological source of energy, Engelhart and Ljubimowa (1939) established that myosin functions as an ATPase. Bruno Straub, working with isolated myosin in the lab of Hungarian scientist Albert Szent-Györgyi discovered that what had been thought of as a single protein, myosin, actually consisted of two proteins, myosin and actin ("actomyosin"), and that actin worked to activate (hence the name) the enzymatic activity of myosin (Szent-Györgyi 2004).

These early studies of the isolated proteins in solution certainly provided important information to researchers. They enabled researchers to identify these proteins, characterize myosin as an (actin activated) enzyme that catalyzes ATP hydrolysis, and identify the conditions which activate or inhibit its enzymatic activity. However, such studies have limitations when the goal is to understand how the enzymatic protein contributes to the production of contractile behavior in muscle cells. There is little obvious connection between these findings and the biological phenomena of muscles contracting. For instance, determining that myosin catalyzes the hydrolysis of ATP shows that it is indeed an enzyme but many molecules are enzymes and not all of them produce contractile movement. To put this in mechanist terms, these studies enabled researchers to identity "parts" that may be parts of the mechanism for muscle contraction—myosin and actin—and to localize an enzymatic "operation" to myosin. But to confirm that these enzymatically active parts are actually the parts of the mechanism *for* muscle contraction, researchers needed to confirm not merely myosin is an actin-activated ATPase but that, as such, it is responsible for producing the contractile behavior of muscle. The development of an IVRS that reconstituted muscle contraction "in a test tube" represented a significant explanatory step (Rall 2018).

In his discussion of this episode, biologist N.I. Arronet (1973) calls the kind of IVRS that researchers developed to understand muscle contraction "cell models" and describes them in terms reminiscent of the "mirroring" relation that Batterman and Rice take to characterize common-features models. According to Arronet, cell models "preserve a high degree of structural orderliness such as characterizes the . . . system *in vivo.*" Cell models can exhibit the relevant contractile behavior while lacking various other elements contained in the *in vivo* system. Ideally, researchers would know that the IVRS contains only the myosin and actin

proteins hypothesized to be the active parts of the muscle contraction mechanism. As I illustrate further with the case study below, if the system is so "biochemically well-defined" and behaves such that it exemplifies the contraction of muscle, then researchers can be confident that they have identified the parts of the mechanism that produces the phenomenon they aim to explain. According to Arronet, cell models can be built in a "top-down" or "bottom-up" manner depending on whether the researchers 1) construct their IVRS starting with whole living muscle fibers and simplify them, leaving the putatively contractile proteins functionally intact or 2) begin with isolated proteins purified from muscle in solution and impose structure on them in an attempt to render the preparation more structurally analogous to how the proteins are ordered *in vivo* (Figure 1).

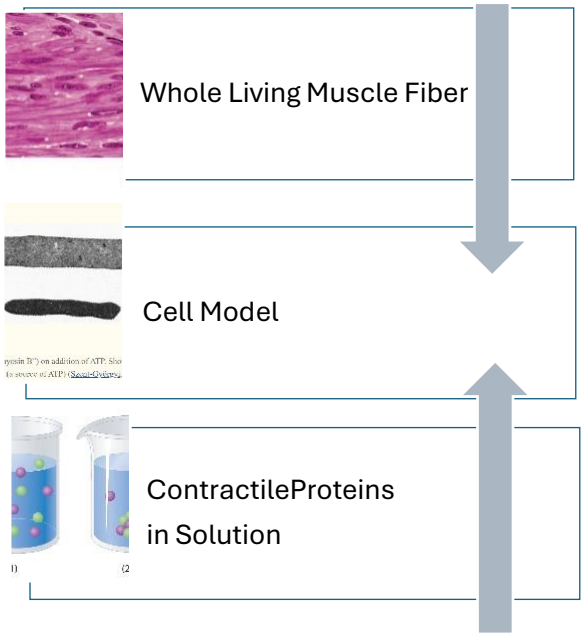

Figure 1.1: Bottom-up and Top-down strategies

To illustrate the "bottom-up" strategy, Moss *et al*. (1935, cited in Engelhardt and Lyubimova 1942) poured myosin in solution onto the surface of a solution of lactate and found that the former formed into 2-D monomolecular films. While these researchers did not use their

preparation for contractile studies, Engelhardt and Lyubimova (1942) reflected on the tenability of such a use. In their discussion, they note a drawback: even if these films could exhibit contractile behavior, they would nonetheless be unsuitable as a model for explaining the contractile system in muscle as the two-dimensional structure of the film "is not typical of the myofibril" in which myosin is organized in long filaments (Arronet 1973 pg. 2). Later researchers attempted to correct for this shortcoming in preparing actomyosin "threads" by compressing such monomolecular films into a filamentous structure.

On my analysis, what Engelhardt and Lyubimova (1942) did was to point out that the IVRS, while perhaps exemplifying contraction, does not exemplify the *explanans* in terms of which muscle contraction is supposed to be explained. In other words, they offer a description of the putative *explanans* in terms of the "typical structure [of myosin and actin] of the myofibril" and insist that, in order for the IVRS to pass muster, it must exemplify contracting muscle at that level. Notice that the failure of this system to exemplify its target (at the level of *explanans*) is not due to a failure of reference. Insofar as they might be put to explanatory purposes, as Engelhardt and Lyubimova suggest, the films would be being *used* as such. As, on Frigg and Nguyen's analysis of exemplification, the reference relation is secured by use, "reference" cannot be the category in terms of which to diagnose the failure of exemplification these researchers pointed out. If, as I do in this analysis, we insist in giving an analysis of in terms of exemplification, that leaves only "instantiation" as the culprit. We must conclude, therefore, that the problem that Engelhardt and Lyubimova point out is that the monomolecular films do not *instantiate* the relevant structure.

Another iteration of such an IVRS is represented in Szent-Györgi's famous actomyosin threads. Following a "bottom up" strategy, Albert Szent-Györgi found that if he extruded a

solution of actomyosin (actin and myosin in solution) from a capillary tube into water, the stream congeals into a gel which forms a thin thread. He found that if placed them into an ATP containing solution, the threads "contracted," like muscle fibers do *in vivo* (Figure 2).

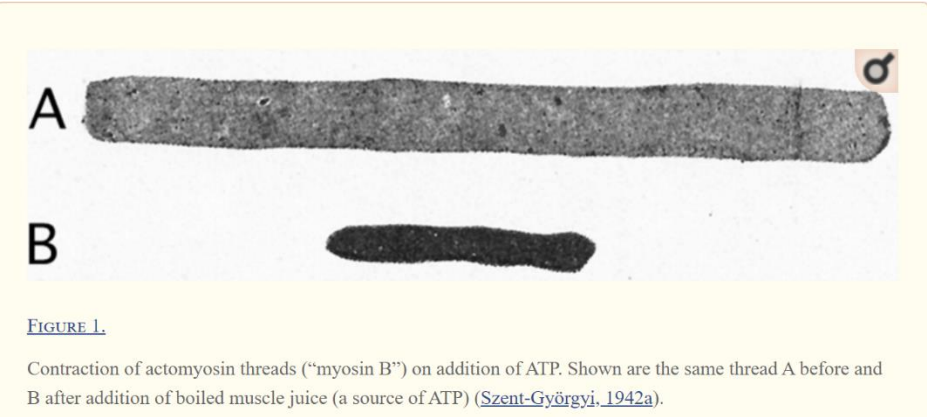

Figure 1.2: Szent-Gyorgyi's contractile threads

This was a striking result (all the more so as Szent-Györgyi was working in total scientific isolation in Hungary during World-War II). Indeed, in his autobiography, Szent-Györgyi writes that, "to see them (the threads) contract for the first time, was perhaps the most thrilling moment of my life" (Szent-Györgyi, 1963). His *in vitro* preparation had given a solution of contractile proteins a degree of structure—representing the "bottom-up" strategy—and found that it "contracted" like muscle fibers do *in vivo.* This "bottom up" research led Szent-Györgyi to declare that the interaction of actomyosin with ATP was "the basic contractile event" (Szent-Györgyi, 2004). Arguably, he had reconstituted muscle contraction "in a test-tube" and, arguably, established that myosin and actin constitute the basic molecular apparatus for muscle contraction.

Once Szent-Györgyi's results became known after the war, other researchers criticized the adequacy of his "threads" as an explanatory IVRSs for muscle contraction. Buchtal *et al*. (1947) found that when they attached Szent-Györgyi's actomyosin threads to a lever, providing the threads with a load on which the contractile substance would presumably pull if indeed it contracted like muscle, the threads actually *increased* in length and their tensile strength *decreased* in contradistinction to the activity of intact muscle. Notice that, in contrast to the

objection raised to the monomolecular films discussed above, this objection is pitched at the level of the *explanandum*—the behavior that the threads exhibited did not instantiate the *in vivo* behavior we are trying to explain by appeal to myosin and actin in building our IVRS.[3] Taking this together with the point made with regard to the monomolecular films, we can appreciate that, for an explanatory IVRS, instantiation must be satisfied at both the levels of the *explanans* and the *explanandum.* This captures what is epistemologically distinctive about the fact that, as explanatory models, IVRSs *must* be made out of the same biological material thought to be involved in the explanatory target. To borrow Frigg and Nguyen's term, they must *literally* instantiate.

As I mentioned above, Frigg and Nguyen (2017) introduce the notion of *I-instantiation* to accommodate their analysis of exemplification to cases of models in which their features do not literally instantiate features of the target. As they write:

> Nothing in what we do with models depends on features being instantiated *literally*. . . [A]n interpretation . . . establishes a one-to-one correspondence of features of the model-object *X* with features of the domain the that the object represents. This correspondence can be exploited to introduce the notion of *instantiation-under-interpreation-I* or *I-instantiation.* . . The idea is simple. The interpretation correlates *X*-features with *Z*-features, and so we can say that the model *I*-instantiates certain *Z*-features iff it instantiates the corresponding *X*-feature. Nothing in our notion of exemplification depends on features being instantiated in a metaphysically robust sense; they can just as well be *I*-instantiated (60).

I-instantiation may be sufficiently general to capture models that both literally and merely "I" instantiate. However, as the example under discussion shows, an explanatory IVRS *must* literally instantiate features of the target system. As the example under discussion shows, an IVRS for muscle contractions aims to identify the molecular-mechanistic causes of muscle

[3] For reasons parallel to those give above in connection with the monomolecular films, "instantiation" is the correct concept to invoke in diagnosing the failure that the researchers are pointing out in this iteration of the IVRS.

contraction *in vivo.* If an iteration were to be constructed that generated contractile behavior *in vitro* but was constructed out of synthetic material, it would lack the bearing that the IVRS is supposed to have on the explanatory hypothesis that it is *literally* myosin and actin proteins driving contraction in the living system.

Finally, because the IVRS is supposed to be *explanatory*, the features at the level of *explanans* and *explanandum* in the IVRS need not only instantiate those observed *in vivo*, the former also need to be identified as *causes* such that the relationship exhibited in the model between the level of *explanans* and the level of *explananda* counts as an explanatory one. To see this, consider Perry, Reed, Astbury, and Spark, (1948)'s criticism of Szent-Györgyi's threads. They compared X-ray images and electron micrographs of myosin and actin filaments taken at different time points during muscle contraction *in vivo* with images of the myosin and actin in Szent-Györgyi's contracting thread taken using the same imaging techniques. As they report:

> Since in an actual muscle both myosin and actin are known to lie lengthways, i.e., parallel to the direction of contraction, the synaeresis of actomyosin in vitro represents a drawing-together in a direction at right angles to the macroscopic change observed in vivo; and therefore, for the present at least, it does not seem possible from electron microscope and X-ray studies to trace any direct relation between the two phenomena (678).

In other words, these critics point out that the characteristic way in which Szent-Györgyi's actin and myosin "draw-together," specifically, at right angles to the axis along which *in vivo* muscle contracts, does not enable us to understand how the microscopic action of myosin and actin observed in the threads (the putative *explanans*) *cause* the macroscopic contractile behavior of muscle (the *explanandum*).

This criticism reveals that, indeed, a mechanistic account of causation is the appropriate one for explicating the way in which an explanatory IVRS aims to explain. If meeting certain

causal theory's criteria for explanation were all that is necessary for an IVRS to be explanatory, the objection under consideration would make no sense. After all, Szent-Györgyi's procedure involved only actin, myosin, and ATP and it showed those elements that are individually necessary and jointly sufficient to cause contraction such that it supports counterfactuals like "if one were to take out any one of those ingredients, contraction would no longer occur." Therefore, the system succeeds in identifying the causes of muscle contraction, at least on this counterfactual dependence analysis of causation. The question of *by what means* do myosin, actin, and ATP cause contraction to occur need not be answered on a such causal theory. But this is precisely the question at issue in Perry et al.'s criticism that close observations of Szent-Györgyi's threads did not illuminate the means by which myosin, actin, and ATP produce contraction. In short, a mechanistic view of causation, one that insists on the necessity of giving an account of the means by which the causal elements identified in an explanatory IVRS produce the phenomenon researchers are trying to explain, is the appropriate notion of causation for purposes of analyzing IVRSs as explanatory models. This perhaps comes as little surprise as mechanistic philosophy of science is rooted in a tradition of drawing on cellular molecular biology for its philosophical insights.

Returning to the objection on which Szent-Györgyi's threads are inadequate on the grounds that they *increase* in length and their tensile strength *decreases* in contradistinction to the activity of intact muscle when exerting force on a load, Szent-Györgyi responded that the concentration of proteins in his threads is considerably less than is found in the myofibril and are likely fragmented rather than composing continuous filaments of myosin and actin like in the intact muscle. With this in mind, Szent-Györgyi reasoned that: "one of the actions of ATP is to enable the actomyosin particles to slip alongside one another. Therefore, if an actomyosin thread

is loaded or subjected to tension, and ATP is added, the actomyosin particles will contract, as they do in muscle, but they will also slip, and in spite of the contraction (observable in unloaded threads), the system will lengthen" (Szent-Györgyi, 1949).

Szent-Györgyi's style of reasoning here gives further motivation for thinking that a mechanist view on explanation is the appropriate one for the epistemological analysis of the relationship between descriptions at the level of *explanans* and *explanandum* in explanatory IVRSs. He is engaging in what mechanists have called "mental simulation" and, by those means, explaining why his threads lengthen upon addition of ATP when attached to a load (Hegerty 2004, Bechtel 2011) Mechanist philosophers of science have appealed to the category of mental simulation in analyzing the explanatory relation between a mechanistic *explanans* and its *explanandum.*[4] Given the low concentration and fragmentation of proteins in his threads, he could mentally simulate how they would, in response to ATP, slip with respect to one another in such a way that the overall length of the thread would increase when attached to a load as observed by Buchtal et al.

What was needed, then, was a means of preparing actomyosin threads which preserved the concentration and continuous filamentous structure found in intact muscle such that the mutual "slipping" of actin and myosin worked to promote, as it did in the intact muscle, contraction under load. He proceeded to develop a preparation that did just that, this time "top down." The preparation involves excising the psoas muscle from a rabbit, carefully separating it into small fiber bundles which were placed into 50% glycerol for twenty-four hours and then 20% glycerol for an hour before further decomposing the fibers into strips of 0.2-0.3 mm.

[4] In fact, we can see mental simulation at work in Perry et als., objection above which can be understood as them claiming that it is not clear how to mentally simulate the production of the contraction along the long axis of a muscle fiber on the basis of the "coming-together" of myosin molecules at right angles to that axis as observed in the threads.

diameter. This technique produced small fibers, free of ATP, but leaving the contractile actomyosin system in the same form as in the living fiber. Many of the properties of the living fiber were eliminated as well including its "selective permeability, excitability, [and] ability to accumulate energy . . ." (Arronet, 1973 p. 7-8). This ensured that the "effects observed on addition of ATP could then safely be ascribed to an interaction of the protein and the nucleotide" (Szent-Györgyi, 1949). The experiments were a success: "If connected to the isometric lever, on addition of ATP they develop tension comparable in intensity to that developed by intact muscle on maximal excitation (Szent-Györgyi, 1949)." This, as Albert's younger cousin Andrew Szent-Györgyi (2004), puts it, "brought conclusive evidence that the interaction of ATP with actomyosin was the basic contractile event."

While Szent-Györgyi's psoas muscle preparation was a landmark achievement, researchers in the following decades noted its shortcomings as a model of the contractile apparatus due to incomplete isolation of the contractile system. Researchers estimated that the model still contained 30% of all noncontractile proteins found in the living muscle fiber (Arronet 1973). Wilson et al. (1959) determined that the cytochrome system remains functional in the fiber model. However, by the mid-1960s, the "top-down" practice of reconstituting muscle contraction *in vitro* was perfected. As Arronet (1973) put it, "[T]he virtually total extraction of noncontractile proteins from fibers while leaving the properties of their contractile skeleton intact" was successfully achieved by Abbott and Chaplain (1966) who found that treating glycerinated insect flight muscle with the detergent Tween 80 eliminated non-myofibrillar enzymatic activity without impairing the contractile action of the fibers.

What this final part of the story illustrates is the importance of an IVRS being "biochemically well-defined." In order for the IVRS to provide justification for the explanatory

claim that "the interaction of actomyosin with ATP is the basic contractile event" it must be such that researchers know all of the elements present in the IVRS. Otherwise, one cannot be certain that the contractile behavior of the IVRS is due to, specifically, the interaction of actin and myosin with ATP and that no other elements are conditioning that behavior. In other words, that one's system is biochemically well-defined is key to justifying that, indeed, the IVRS has identified the causes on which the behavior of the system counterfactually depends. Of course, as I pointed out above, this alone is not sufficient as researchers develop IVRSs for purposes of understanding the means by which those causes produce or generate the phenomenon of interest. For this reason, a mechanistic account of explanation is the appropriate one for the epistemological analysis of explanatory IVRSs. The level of *explanans* is formulated in terms of the *explanans* that count as mechanistic causes for the *explanandum* behavior of the system.

In sum, the epistemological constraints on developing an explanatory IVRS for muscle contraction are as follows:

- Features of the IVRS must literally instantiate (as opposed to merely I-instantiate) and, therefore, literally exemplify features of the target as observed *in vivo*, at both the level of *explanans* and *explanandum*.
- The IVRS must identify the causes of the *explanandum* and the means by which those causes produce it.
- Comparisons between the IVRS and the modeled target as observed *in vivo* drive the iterative process of producing an IVRS that meets these constraints.

Section 3

As the case described in the following section shows, the practice of developing a *measurement* IVRS overlaps with but importantly departs from the modeling practice described above. Regarding the overlap, in developing a measurement IVRS 1) it is important that the system be biochemically well-defined, 2) researchers can pursue either top-down or bottom-up strategies. The epistemological *telos* of the practice, however, is not that of exemplifying a particular *in vivo* phenomenon of explanatory interest. Rather, it is that of exemplifying the quantity that researchers aim to measure and exemplifying it in a way that makes it accessible to be measured. Again, as in the case of explanatory IVRSs, instantiation here must be literal. However, the significance of the system's being biochemically well-defined differs across purposes of explanation and measurement. In the measurement practice, it serves to justify that the signal that shows up observably—thus making the quantity accessible to measurement—in the IVRS is produced by and only by the quantity they aim to measure. On the basis of this fact, researchers can justify their claim to have built an IVRS that exemplifies that quantity. The following case study provides an illustration. Before jumping into the case, however, I need to do some historical stage-setting.

The research discussed above, notably that of Szent-Györgi, set the stage for A.F. Huxley and Neidergerke's (1954) and H.E. Huxley and Hanson's (1954) landmark observations of thick and thin filaments in the muscle sarcomere using X-ray interference and electron microscopy. This work led H.E. Huxley to develop a "sliding filament" model of muscle contraction. On this picture, actin and myosin in the muscle sarcomere (the basic functional unit of muscle cells)

form interdigitating filaments which slide past one another, in an ATP dependent manner, with myosin working as the enzyme (Figure 3).[5]

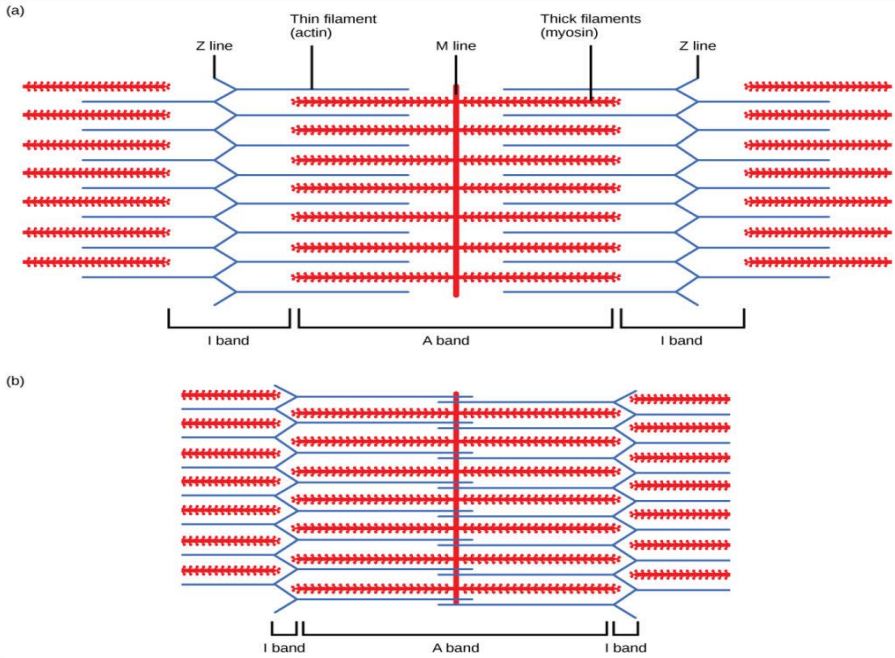

Figure 1.3**:** Interdigitating filaments of myosin (red) and actin (blue).

Shortly afterwards, H.E. Huxley (1969)developed an account based on his observations of "crossbridges" between thin (actin) and thick (myosin) filaments. These led him to propose that the sliding is driven by "swinging cross bridges" in which myosin cycles through stages in which it detaches from actin (1), moves (2), reattaches to actin (3), and exerts force in a "powerstroke" to pull the actin filament along (4). (For detailed historical accounts, see Needham, 1971;

[5] The fact that myosin turned out to be enzymatically active was quite a surprise. Myosin is a structural protein, it performs its function as a *filament* composed of many individual myosin strung together by the light chains at their C-terminal ends. The dogma of the day was that only soluble proteins—rather than structural ones—were enzymes.

Huxley, 1977; Rall, 2014). Altogether, the myosin heads function like oars on a Roman galley with the actin serving as the water.

Later, biochemists Lymn and Taylor (1971) mapped the steps of myosin's movement onto the steps of the molecule's hydrolytic cycle, proposing what came to be known as the Lymn-Taylor cycle (Figure 4). First, myosin binds ATP and detaches from actin (1). As ATP is hydrolyzed the crossbridge returns to a right angle (2); it then binds to a new locus on myosin (3). Myosin remains bound to the reaction products (Pr), ADP and Pi, until step 4, at which their release corresponds to a movement of the crossbridge.

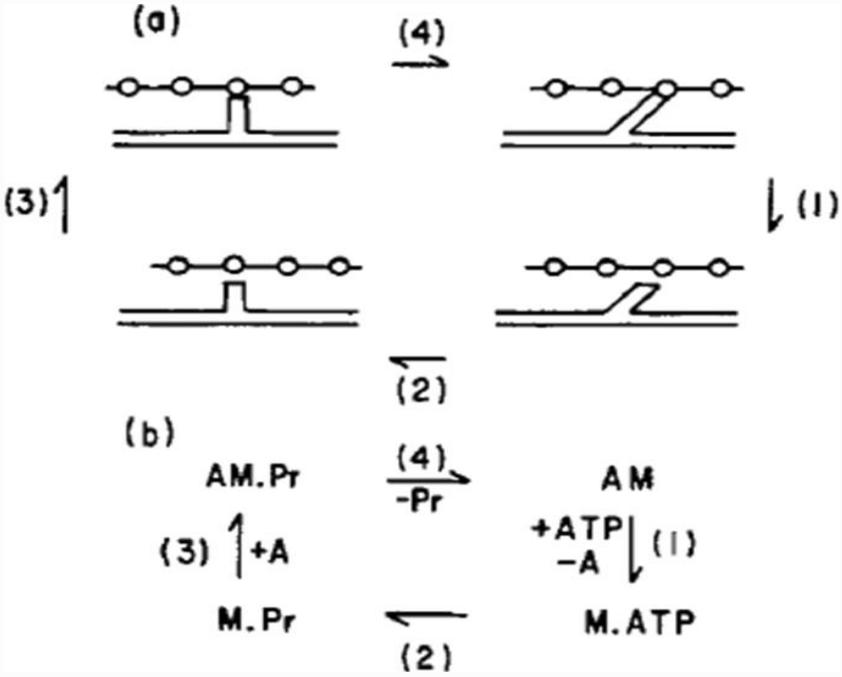

Figure 1.4: The mechanical movement of the myosin "crossbridge" as it attaches to and releases from myosin coupled with the enzyme's chemical cycle.

In addition to shedding important light on the mechanism of muscle contraction, this work—especially Huxley's development of the sliding-filaments model—influenced thinking about a number of other cellular motility phenomena of interest to researchers in the mid-20th century. By "cellular motility phenomena" I mean a class of phenomena characterized by

movement including both movement of cells and things in them.[6] The simplest motility phenomenon is *Brownian motion* of small particles produced by thermal forces from neighboring molecules. "*Saltatory" (or jumping) motion*, on the other hand, is the directional movement of particles over distances too large and at rates too fast to be accounted for in terms of thermal forces. It may involve changes in particle velocity, sudden stops, and changes of direction.[7] Other motility phenomena included *cytoplasmic streaming* (the intracellular flow of the viscous fluid inside of cells), *ameboid movement* and *ciliary and flagellar movement*. The mechanisms responsible for such movements remained a mystery in the mid-20th century.[8] Muscle contraction was the first such phenomenon to receive a consensus-generating treatment—the one described above—but it was certainly not the only one that researchers suspected to involve myosin and actin. Indeed, it is difficult to overstate the influence that the picture of sliding myosin and actin filaments in muscle exerted on mid-20th century studies of these other cellular motility phenomena.

Although there was little direct evidence for the existence of such supramolecular structures in non-muscle cells, the picture of interdigitating actin and myosin filaments inspired the development of mechanistic models to explain other non-muscle motility phenomena. In (Figure 5, top) below, we see (Huxley 1973)'s "sliding filament" model of the mechanism for cellular locomotion. (Spudich 1974) speculated that actin and myosin may be involved in vesicle transport as well (Figure 5, bottom). These models took the basic structure of the mechanism for muscle contraction and applied them to motility phenomena in non-muscle cells. As (Sheetz and

[6]See Editor's Introduction to: Allen, R.A and N. Kamiya (1964).
[7] For relevant philosophical work on saltatory motion—axonal transport in particular—see Matlin, K. S. (2020) and Bollhagen (2021).
[8] The motor driving ciliary movement, dynein, was discovered in 1963. See Gibbons (1963).

Spudich 1983) write, " . . . models of motility in non-muscle cells have substituted membranes for the Z-lines in muscle, and the myosin is shown in the form of bipolar filaments" (485).

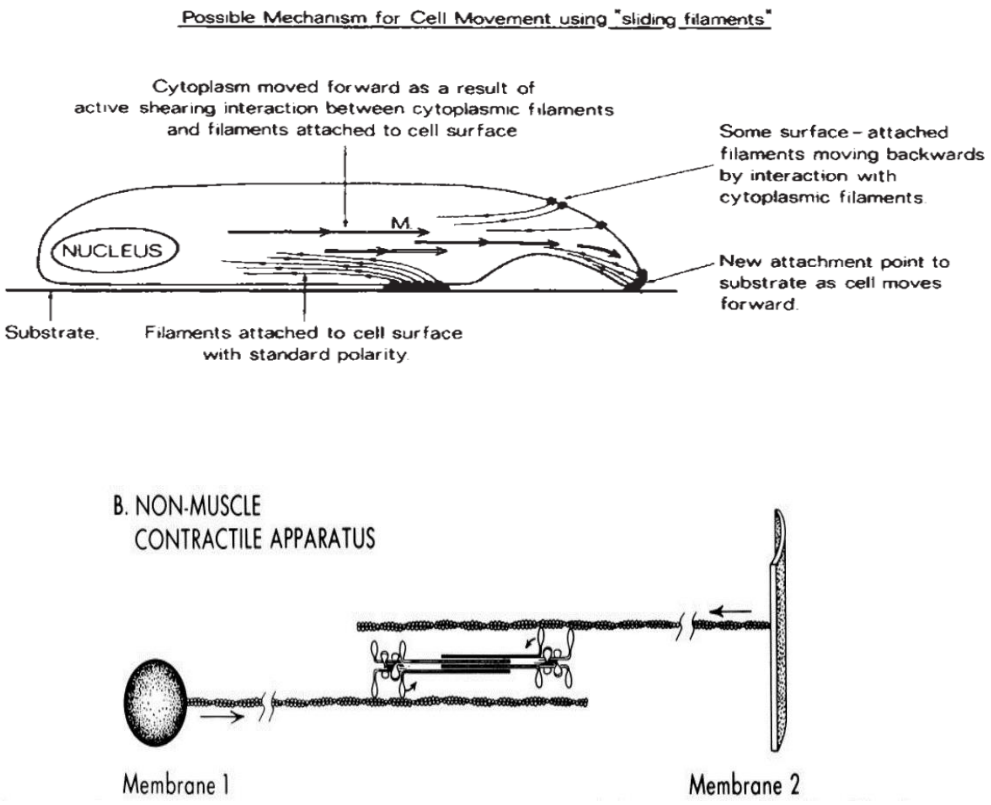

Figure 1.5: sliding-filament models for cell movement. Image from (Huxley, H.E., 1973) (Bottom) a sliding-filament model of saltatory particle movement. Image from (Scheetz and Spudich 1983).

So, this was the scene in the mid-1970s when James Spudich was heading up a laboratory in the Structural Biology Department at Stanford. Spudich set two goals for his lab: 1) "to understand the various forms of motility observable in non-muscle cells" and to do so in part by 2) "develop[ing] a biochemically well-defined *in vitr*o reconstituted system for myosin movement on actin" (Spudich, 2011). As Spudich's graduate student, Stphen Kron put it, the

IVRS assay they aimed to design would be "a system which would permit quantitative determination of the rate of myosin movement along actin" (Kron 1990, 32). In other words, their purpose was to create an IVRS for purposes of *measurement.* To this end, researchers iteratively produced a system which (literally) exemplifies *the characteristic rate at which myosin moves over actin.* Let us turn to the case study.

Spudich's first attempt was a clever application of the "top-down" strategy I described above. As depicted in (Figure 6), it was known at the time that actin filaments could be found in the cortex of the phagocytic (meaning it engulfs and consumes foreign material) *Dictyostelium* (Ishikawa *et al.*, 1969; Schroeder, 1973).

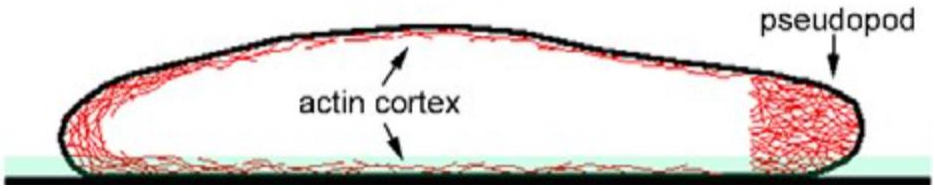

Figure 1.6: Actin in the cortex of *Dictyostelium.* Image from: Yumura (2012).

By feeding *Dictyostelium* polystyrene beads and isolating the phagocytic vesicles that formed around them, he was able to recover the beads, coated in cell membrane, with actin filaments emanating outwards (Figure 7).

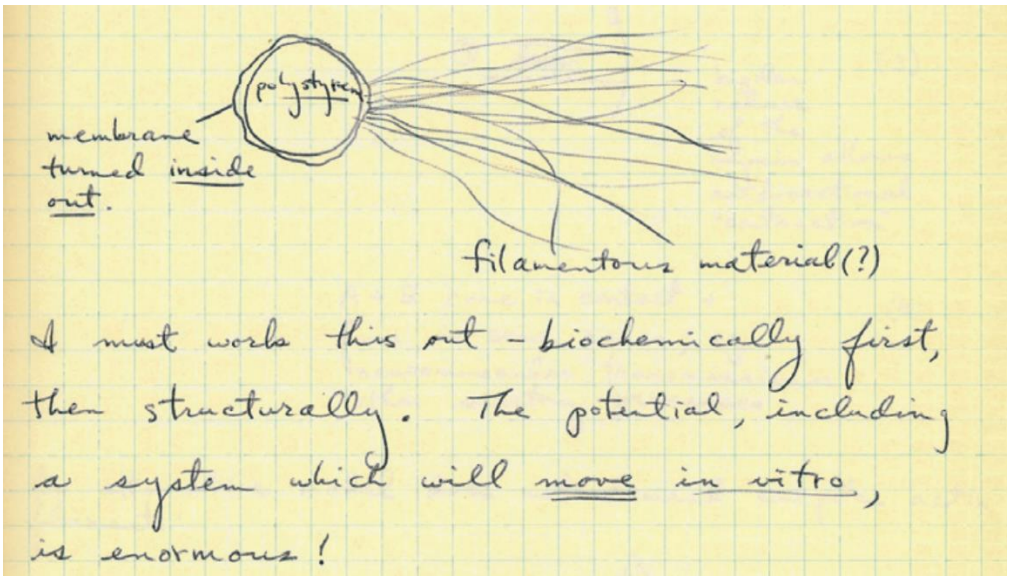

Figure 1.7: Spudich's lab note. Image from (Spudich, 2012).

Using these beads, Spudich attempted to reconstitute motility by putting them on a myosin-coated glass coverslip and adding ATP but to no avail—no observable motion at all was produced, much less motion that would exemplify the rate at which the unobservable myosin moves over actin (Spudich 2011). Note, however, a further problem. Even if motion had been apparent, the system was not biochemically well-defined—it contained whatever other elements might be present in *Dictyostelium* cytoplasm—which would undercut confidence that the hypothetical observed motion was due to the action of myosin moving on actin, specifically. What we would have then is a system akin to early iterations of the psoas muscle preparation discussed in the previous section in which contractile behavior was observable but the fact that elements other than myosin and actin might be operative limited researchers confidence that it was myosin and actin specifically driving that contractile behavior. Indeed, as I pointed out above, it is generally important to the successful development of a reconstituted system that it be biochemically well-defined. Importantly, however, the fact that this measurement system was not

biochemically well-defined is of different epistemological significance. Rather than the system's failure of biochemical well-definition representing a failure to identify the causes of a target of explanation, here that failure represents a failure to exemplify the quantity they aim to measure—the characteristic rate at which, specifically, *myosin* moves over *actin.* If the system is not biochemically well-defined, researchers cannot be confident that only actin and myosin are producing that observable movement and, therefore, cannot be sure that it literally instantiates the target rate.

Spudich's next attempt was to pursue a "bottom-up" strategy. "Flipping the geometry" of his initial experiment, instead of coating beads with actin and coverslips with myosin, Spudich and his lab-mates coated beads with purified myosin and bound purified actin filaments to slides, anchoring them at their barbed ends, and running an aqueous solution over them in hopes of orienting them in the direction of the flow.[9] The hope was to get an array of well-oriented purified actin cables along which myosin-bound beads would move. Spudich and colleagues deposited the beads in the preparation but, while some motion was observable, it was difficult to generate bead movement across attempts and, when they did get it, the movement was sporadic at best. Spudich's diagnosis: "In retrospect, we probably did not have sufficient alignment of filaments; we were not monitoring filament alignment at that time by electron microscopy, as we did later" (Spudich 2012).

Note that this attempt, unlike Spudich's prior one, used purified actin filaments and beads coated with purified myosin. Thus, assuming their purification procedures were up to snuff, the system was biochemically well-defined and, so, they could be confident that whatever bead

[9] To get an intuitive sense of this technique, imagine tying ropes onto a rocks in the middle of a flowing river, one rope per rock. The ropes will extend outward, their free ends oriented away from the rocks on which they are secured, in the direction of the water's flow.

motion they were generating was being driven by the movement of myosin over actin. So, the fact that this iteration was judged a failure is not due to its not being biochemically well-defined but, rather, due to the fact that the motion they managed to produce was not suitable for their purposes. Again, that purpose is to measure the characteristic rate at which myosin moves over actin. To that end, they aimed to create an IVRS that would exemplify that rate. The movement they observed was, they could be confident, *driven by* the movement of myosin and actin but, being sporadic and irregularly produced, it did not exemplify the *rate* of the latter movement.

Frigg and Nguyen (2022) offer another way of understanding this point. Consider again a blue paint chip. For purposes of using a blue paint chip in the way you would if you were looking to paint your living room, it would not be sufficient for it to *merely* instantiate the relevant shade of blue. "A colour swatch that's too small to see with the naked eye does not, in the context of a paint shop, exemplify royal blue, even if the colour could, in principle be seen under a light microscope. An exemplar is therefore not merely an instance of a feature but a *telling instance*" (60).

Because this iteration of their measurement IVRS was biochemically well-defined, Spudich and colleagues could be confident that the motion they were observing was an instance of, specifically, *myosin-actin* driven motion. It was not, however, a "telling instance" that exemplified the rate at which myosin moves over actin.

Things started to change when Mike Scheetz joined Spudich at Stanford in 1982. Scheetz realized that Nature could provide sufficient alignment of actin filaments. A decade prior, researchers studying cytoplasmic streaming found uniformly oriented bundles of polar actin filaments in the cortex of the internodal cells of *Nitella*, a green algae (Pavlovitz, *et al*., 1974; Kersey *et al*., 1974; Kersey *et al*., 1976). Knowing this, Sheetz pursued a "top-down" approach.

He reasoned that if he could cut open vertically a length of the cylindrical *Nitella* cell, open it up to form a sheet, and pin it flat to a slide, actin-side up, he would have a surface of nicely oriented actin filaments on which to place myosin coated beads (Figure 8).

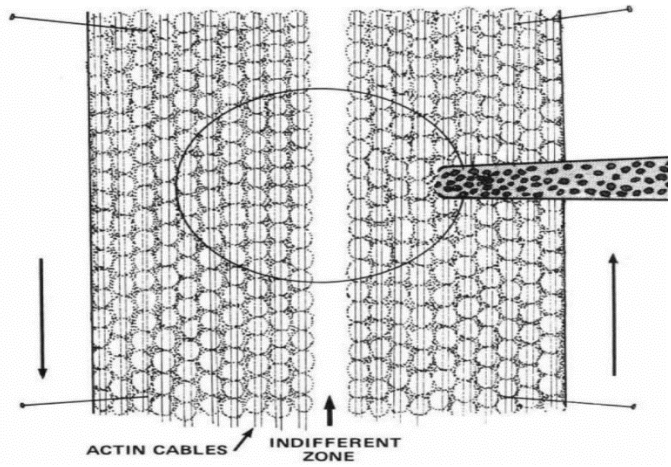

Figure 1.8: The internodal cell of *Nitella,* sliced open vertically and laid flat. Image from (Scheetz and Spudich, 1983).

Notice that, as in the *Dictyostelium* preparation discussed above, the choice to use *Nitella* was not guided by an interest in understanding how actin worked with myosin to drive any particular cellular activity exhibited in the *Nitella* cell. Unlike in the case of an explanatory IVRS these researchers were not interested in explaining anything about the biology of *Nitella*. Rather, the algae was chosen as a convenient way of getting a well-aligned lawn of actin filaments on which to make myosin coated beads move observably and, with hope, more reliably and less sporadically.

The *Nitella* system indeed represented a turning point. Adding myosin-coated beads to the exposed actin fibers produced robust, ATP-dependent, unidirectional movement in the direction of the left and right-most arrows in the figure above. To the left of the indifferent zone,

beads moved “downwards” from the pointed end of the actin filaments toward the barbed ones. On the right of the indifferent zone, where the pointed ends are at the bottom of the diagram, the beads moved “upwards” toward the barbed ends. Perfecting this version of the assay, these researchers refined their methods for preparing the beads, determining the ideal amount of myosin with which to coat them and the ideal buffer conditions for generating maximally robust, stable movement. They published a report of their system in *Nature* in May of 1983. As the authors put it, this assay constituted the “first direct visualization of myosin movement on actin” observing, among other things, that the velocities of the beads “range over 0.5-10μm/s (average 2.5μm/s)” (Scheetz and Spudich, 1983). In terms of the present analysis, the assay literally exemplified the characteristic rate at which myosin moves over actin, at least arguably.

However, insofar as it was not a biochemically well-defined system, the claim that it exemplified the rate at which, specifically *myosin* moves on *actin* was contentious. Indeed, much of the argumentation in the *Nature* paper is designed to justify Scheetz and Spudich’s claim that their system was measuring what they were aiming to measure. Reflecting on their arguments is interesting as they involve drawing comparisons between the rate of movement observed in their IVRS and the rate at which myosin-driven actin motion occurs in *in vivo* systems. In the modelling practice, as I described above, such comparisons drive a bootstrapping procedure which renders iterations of the system increasingly exemplary of the explanatory target at both the level of *explanans* and *explanandum*. But in the case under discussion presently, these comparisons reflected their commitment to what Hasok Chang (2009) refers to as the “principle of single value.”. These researchers were developing an IVRS for purposes of measurement and, thus, it should come as little surprise that what Chang identifies as the

”ontological principle” to which one must commit in order to intelligibly pursue the “epistemic activity” of measurement is the one to which researchers in my case committed.

Let us consider their arguments. First, the authors point out that, “if the . . . bead movement is driven by the myosin head groups, ATP should be required and specific inactivation of the myosin heads should block movement” (33). Indeed, removing ATP from the preparation stopped the beads from moving. Also, it was known that myosin treated with NEM (N-ethylmaleimide) will bind to actin but not release from it even in the presence of ATP. NEM treatment of the beads did indeed stop the motion indicating that the motion of the beads was in fact driven by the movement of myosin over actin. This argument is meant to shore up confidence that the movement exemplifies the rate at which, specifically *myosin* moves over *actin* in spite of the fact that, even given the results of this control experiment, it remained possible that other elements might be conditioning that movement.

Second, Scheetz and Spudich compared the motion of the beads to that found in *in vivo* systems. Immediately following the reports of their measurements, the authors state that the velocities of the beads “range over 0.5-10μm/s (average 2.5μm/s); *these are comparable with the rates of relative sliding of myosin and actin filaments in muscle* (my emphasis).” In addition to drawing this comparison with respect to the rate of myosin and actin sliding in muscle, the authors pointed out that the *directionality* of the bead’s motion was consistent with that of the motion produced by actin and myosin in the muscle sarcomere during contraction. During contraction, myosin moves toward the barbed end of actin filaments just as the beads moved toward the barbed ends of the *Nitella* cables in the *in vitro* system. Also, the streaming of cytoplasm in the *Nitella* cell prior to dissection was observed to proceed in the same direction as did the beads when they were introduced into the preparation—“upwards” or “downwards”

depending on the orientation of the actin proximate to the movement: ". . . beads observed *in vitro* on both sides of the indifferent zone move in opposite directions, and the directions are the same as those of the cytoplasmic streaming *in vivo"* (33). On the basis of such comparisons, they argue that "Thus, movement of beads occurs with the expected characteristics of a myosin driven process; it requires contact of the beads with the actin cables and proceeds in the proper direction" (33).[10]

In a series of experiments using this assay but in which beads were coated with myosin derived from different sources (*Dictyostelium,* skeletal muscle, smooth muscle), (Sheetz, Chasan, and Spudich 1984) found that "Different myosin species move at their own characteristic velocities, . . . and further, the velocities of the beads coated with smooth or skeletal muscle myosin correlate well with known *in vivo* rates of myosin movement along actin filaments in these muscles" (1867). Again, in this follow-up paper, we see the authors comparing the velocity of the motion observed in their reconstituted motion with that of independently determined rates of motion *in vivo* and concluding that, "This *in vitro* assay*,* therefore, provides a rapid, reproducible method for quantitating the ATP-dependent movement of myosin molecules on actin" (1867). Again, as this last quote makes clear, the goal in developing their reconstitutes system was *measurement.*

In comparing the measured rate of movement observable in their system with independent measures of myosin/actin driven motion in other systems, these researchers pursued an "epistemic activity" that Chang (2009) refers to as *testing-by-overdetermination.* Pursing

[10]Here is another argument given in defense of the assay in the same paper: "The major weakness of the assay is that the Nitella substratum is not biochemically defined. There could be trace amounts of Nitella enzymes or proteins that modify the myosin and affect its motility However, we have no results to date to suggest that this complication is a serious one. The cytoplasmic contents of the Nitella cell are diluted 2,000- 4,000-fold in the dissection buffer, which significantly reduces the probability of enzymatic modification during the course of the assay" (1870).

testing-by-overdetermination requires a "conditional commitment" to what Chang (2009) calls the *principle of single value.* Here is Chang, "The principle states that a real physical property can have no more than one definite value in a given situation; or, that two correct value ascriptions about the same situation cannot disagree with each other when we are concerned with a real physical property" (68). In testing-by-overdetermination "we determine the value of a quantity in two different ways. If the values match, that gives credence to the basis on which we made the two determinations; if the values do not match, then we infer there is some problem with our starting assumptions. The two determinations could be the familiar prediction-observation pair, or two observations, or even two theoretical determination." (70). In the case I discuss, the "determinations" were, specifically, the rate of the observable movement produced by the movement of myosin over actin in various systems including the IVRS. The argument is that if these rates correspond, researchers can be more confident that the rate of the observable movement in their IVRS exemplifies the characteristic rate at which myosin moves over actin.

Let us step back and appreciate this point in the wider context of my analysis. Above, I distinguished between top-down and bottom-up approaches to building reconstituted systems. In pursuing a bottom-up approach in which researchers start with purified proteins, the goal of building a biochemically well-defined system is easily met; trivially so, in fact, assuming that the purification procedures used are up to snuff. Taking a top-down approach, as Spudich *et al.* did in their *Nitella* system, comes with more liability. It is considerably more difficult for researchers to be confident that, in an iteration of such a system, no other elements are active in determining the character of its observed behavior. One way to cope with this problem is to develop further iterations of the system. However, if the system is apparently producing compelling data, as the *Nitella* system was, researchers may test-by-overdetermination in order to formulate arguments,

the likes of which we just saw, to defend the system as an adequate instrument of measure in spite of its not being biochemically well-defined. This aspect of the practice is expressive of the way in which researchers are committed to the principle of single value in developing measurement IVRSs.

However compelling these arguments may have been, not everyone was convinced. In the Fall of 1983, a first-year graduate student, Stephen Kron, listened to Spudich lecture about the *Nitella* assay in a graduate course. He was concerned about the crudeness of the preparation.[11] Although the arguments explicated above might raise confidence that it was myosin and actin producing the motion, insofar as the goal of developing a biochemically well-defined system had not been achieved, that confidence derived merely from indirect argumentation, not direct demonstration. Kron went to Spudich's office after the lecture and, after sharing his concerns and thoughts about how to improve the assay, Spudich asked him to join the lab and lend a hand. Kron joined Spudich in January of 1984.

Kron's first effort was to revisit the "bottom-up" design involving purified actin filaments anchored to glass cover-slips. This time, however, Kron would observe them under electron-microscopy to ensure that the filaments were indeed in the proper alignment and orientation. Even with the aid of EM, though, it was difficult to get a well-aligned lawn of actin filaments and, running the assay, he found that "many beads attach to the substratum without moving, and those that move do so for relatively short distances" (Kron, 1990 p. 33).

Seeking to develop an approach which would remove the requirement for a large array of actin filaments, Kron was inspired by observations made by Yanagida et al. (1984) who

[11] In his words, Kron "was one of many who couldn't believe that Nature paper was even published . . . In fact, when Jim lectured on the Nitella assay to the grad class I was taking in the fall of my first year, I walked down after his lecture and told him that he was way over-interpreting his results (who knows what was in there?)." Personal correspondence.

developed a technique that enabled the visualization of fluorescently labeled actin filaments moving in the presence of myosin. The movement Yanagida and colleagues' analysis focused on, however, was the *bending* of actin filaments. They hypothesized that when myosin heads bind to actin, they produce local distortions in the actin proteins resulting in the observed bending of the actin filaments.

Taking a cue from this research, Kron immobilized myosin filaments to glass coverslips and labeled single actin filaments with rhodamine phalloidin, rendering them visible under fluorescence microscopy. He wanted to see whether immobilized myosin filaments would support the sliding (rather than bending) of actin filaments. They did indeed. Not only did the actin filaments slide but their movement was long-lasting, with a single preparation producing continuous recordable movement for over an hour. Further, in a series of experiments varying the species from which the myosin and actin were derived and measuring the rates of movement produced by each combination, Kron (1990) determined that "the major determinant of the rate of movement of actin and myosin is the type of myosin" (40).

Kron also ran the experiment with a very low concentration of myosin filaments immobilized onto the glass coverslip. As might be expected, only occasionally did he observe an actin filament to bind to immobilized myosin and move along the surface of the glass. However, during their motion, these actin filaments would break less frequently than they did when moving across a coverslip coated more densely with myosin filaments and the distance of the movement was equal to or less than the length of a single myosin filament. These observations suggested that the actin filaments were moving along *single* myosin filaments and that, therefore, "the movement of actin over myosin requires at most the number of heads in a single thick filament" (40).

At this point, there was no question that the rate of the movement observable in this system (the rate of the actin filaments) exemplified the unobservable characteristic rate at which myosin moves over actin. Not only was the reconstituted system biochemically well-defined, removing all doubt that elements other than myosin and actin might be involved in the observed sliding motion but, additionally, there was no further object, like the beads in prior iterations of the system, the movement of which was supposed to indicate, indirectly, the movement of myosin on actin. This was, so to speak, the straight dope. This version of the reconstituted system therefore enabled the measurement of the characteristic rate at which myosin filaments move on actin, which was found to be 3-4 μm per sec for skeletal muscle myosin and 1-2 μm per sec for *Dictyostelium* myosin.

It was not only measurement of the characteristic rate at which myosin moves over actin that pursuing this measurement IVRS research enabled. In the following decades, a number of increasingly sophisticated versions of the actomyosin reconstituted system were developed that exemplified and, therefore, enabled the measurement of the characteristic "step-size" of a single-myosin head as it moves over an actin filament, and the characteristic force generated by single myosin molecules. Researchers used laser traps to manipulate beads attached to each end of an actin filament (Figure 9). The actin filament was lowered onto a silica bead and was displaced observably by the action of a single myosin molecule mounted atop the bead. On the basis of these displacements, researchers concluded that a single myosin molecule steps, on average, 11nm over actin and exerts a force of approximately 3-4 pN (Finer *et al.,* 1994).

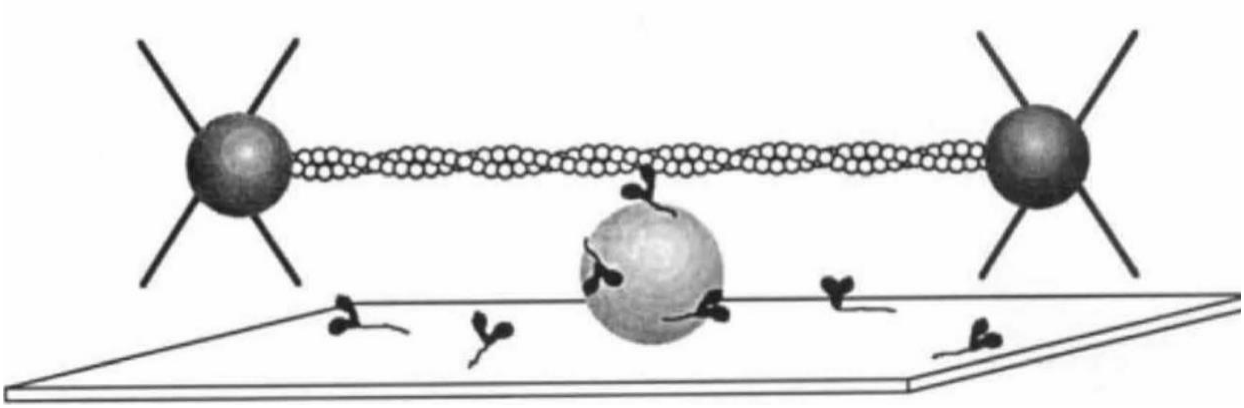

Figure 1.9: Measuring the step-size and force generated by single myosin molecules. Image from (Spudich 2012).

I concluded the last section summarizing the epistemological constraints for explanatory IVRSs. I reproduce them here to facilitate comparison with a comparable summary for measurement IVRSs.

Explanatory IVRS:

- Features of the IVRS must literally instantiate and thereby literally exemplify features of the target as observed *in vivo*, at both the level of *explanans* and *explanandum*.
- The IVRS must be biochemically well-defined in order to identify the causes of the *explanandum* and the means by which those causes produce it.
- Comparisons between the IVRS and the modeled target as observed *in vivo* drive the iterative process of producing an IVRS that meets these constraints.

Measurement IVRS:

- The IVRS must literally instantiate and thereby literally exemplify the quantity to be measured.

- The IVRS must be biochemically well-defined in order to be confident that the observable quantity instantiates the quantity to be measured.
- Comparisons between the IVRS and systems observed *in vivo* reflect that the practice of building a measurement IVRS involves a commitment to the principle of single value.

Conclusion

In this chapter, I have compared and contrasted the iterative practice of building an explanatory IVRS with that of building an IVRS for purposes of measurement. In both practices, exemplification plays an important role. In constructing the IVRS to explain muscle contraction, and in constructing the IVRS to measure myosin movement, researchers aimed to build a model that "exemplifies" their modeling target. The means by which this aim is achieved, however, differs depending on whether the IVRS is being constructed for purposes of explanation or measurement. Specifically, in the explanation case, researchers iteratively develop their system to exemplify a biological phenomenon studied by other researchers using other tools and techniques. This other research provides the concrete standards by which researchers evaluate iterations of their IVRS. This iterative practice is "exemplification" in this sense of Nersessian (2022). I illustrated this practice by means of the case studies I described above. It is aimed at developing a system that does in fact exemplify—in the sense of the abstract relation analyzed in terms of reference and instantiation—the biological phenomenon they aim to model. Comparisons between iterations of their IVRS and their modeling target as characterized by other researchers both at the level of *explanans* and *explanandum* therefore, drive the iterative

practice of developing an IVRS to explain a particular biological phenomenon. These comparisons are made to ensure that the explanatory IVRS exemplifies the explanatory target at both the level of *explanans* and *explanandum*.

For the case of the measurement IVRS in this section, however, the categories of *explanans* and *explanandum* are analytically irrelevant for the simple reason that the researchers built it for purposes of *measurement*, as opposed to explanation. Unlike in an explanatory IVRS, a successful measurement IVRS need not exemplify any particular *in vivo* phenomenon, neither at the level of its outward behavior nor at the molecular mechanical level. What is significant is that it exemplifies the quantity researchers aim to measure. To the end, its outwardly observable behavior must be such that it can serve as an indicator on the display of a measuring device. It is also important here, as it is in the case of explanatory IVRSs, that the system be biochemically well-defined. However, rather than the fact of its being biochemically well-defined serving to confirm that the parts of the IVRS are indeed parts of the relevant explanatory mechanism, in the measurement case, it serves to justify that the signal that shows up observably in the IVRS is produced by and only by the quantity they aim to measure. In this way they can justify their claim to have built an IVRS that exemplifies that quantity.

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Chapter 2: The "Inchworm Episode": Reconstituting the Phenomenon of Kinesin Motility

Introduction

Following (Bogen and Woodward 1988), the New Mechanist philosophy of science tells us that *phenomena* are targets of explanation in science. Traditionally, in this school, philosophical focus has been on the analysis of explanation, leaving phenomena construed as little more than the targets thereof. Familiarly, mechanistic explanation consists in specifying the organized parts and operations (entities and activities) constituting the mechanism responsible for generating a phenomenon of interest (Bechtel and Abrahamsen 2005; Machamer, Darden and Craver 2000). As (Illari and Williamson 2015) put it:

> All mechanistic explanations begin with (a) the identification of a phenomenon or some phenomena to be explained, (b) proceed by decomposition into the entities and activities relevant to the phenomenon, and (c) give the organization of entities and activities by which they produce the phenomenon (123).

However, philosophers have recognized that this gloss on the research process is overly simplistic since (Bechtel and Richardson 1993/2010) coined the phrase "phenomenon reconstitution" in their seminal work on mechanistic research.[12] Mechanists observe that researchers frequently re-understand an initially identified phenomenon as they acquire insight into the mechanism(s) responsible for it. Mechanist philosophical models of how phenomena are reconstituted in science tend to emphasize the importance of explanatory considerations in driving the process. On such models, phenomena are reconstituted as researchers gain insight into the explanatory mechanisms underpinning phenomena of interest (Bechtel and Richardson

[12] While the term "reconstitution" may have Kantian connotations for some readers, as it is used by these authors (and myself) no such connotations of the term are intended.

1993/2010; Craver 2007), or as researchers recognize that their favored explanans is better suited to explain a phenomenon occurring at a "level of abstraction" higher than was initially assumed (Kronfeldner 2015). This emphasis is perhaps unsurprising given mechanists' traditional focus on explanation. That said, a number of philosophers have recently considered the ways in which scientists treat phenomena as objects of investigation in their own right (Colaço 2018; 2020; Feest 2011; 2018). Taking cues from this recent work, I analyze a case of phenomenon reconstitution that occurred entirely within an experimental program dedicated to characterizing, rather than explaining, the phenomenon of kinesin movement.

Research on kinesin—a molecular motor that transports cargo around cells by moving unidirectionally along microtubule protofilaments—involves a substantial amount of experimental work dedicated to characterizing the phenomenon of kinesin movement. Unlike with macroscopic objects whose movements are readily observable, molecular motor movement is a phenomenon that takes place at the nanoscale. Characterizing it therefore presents challenges that require sophisticated experimental tools. In what follows, I focus on a particular tool, the *single-molecule motility assay*. Like patch-clamp recordings that made possible the single molecule investigation of ion channels in neuronal membranes, the single-molecule motility assay enabled researchers to study the kinetic activities of single kinesin molecules and was an invaluable tool in the effort to characterize kinesin movement.

That the appropriate characterization of kinesin movement is that it walks "hand-over-hand" along microtubules was a guiding idea for researchers using the single-molecule motility assay.[13] In fact, the hypothesis was first suggested in 1989 in the very article reporting the

[13] This idea guided researchers using other methods as well, in particular, those using traditional biochemical techniques to study the hydrolytic cycle of the kinesin molecule. The interactions between the biochemical and single-molecule programs was important in the effort to map the stages of kinesin's mechanical steps to stages in its hydrolytic cycle. Further, biochemical work showing that the hydrolytic state of one head limited the hydrolytic

development of this experimental tool. Over the following ten years, data from studies using variations on the basic design of the assay were interpreted as supporting hand-over-hand (HoH) walking, generating a limited consensus that, indeed, the correct characterization of the phenomenon of kinesin movement was that it walked HoH.

However, in 2002, a study involving a particularly interesting variation on this assay briefly disrupted this consensus, making a compelling case that kinesin walks in an "inch-worm" fashion rather than HoH. This study was quickly followed by a number of further single-molecule studies that re-established an even more robust HoH consensus. However, this is not a story of HoH advocates having been correct all along. Rather, the phenomenon of HoH walking was importantly "reconstituted" across the 2002 study.

Section 1 situates the analysis of the Inchworm Episode presented in Sections 2-4 in the context of the broader philosophical discussion of phenomena and provide an indication of how I understand "phenomena" and "phenomenon reconstitution" for the purposes of the analysis to follow. In order to let the case speak for itself as much as possible, I forgo further philosophical discussion until the final section. In Section 2, therefore, I turn directly to the science. I discuss the initial battery of single-molecule studies that were taken to support the HoH characterization of kinesin motility paying particular attention to the empirical criteria—*processivity* and *coordinated head activity*—in terms of which that characterization was specified, that individuated the HoH characterization as such and informed researchers' interpretations of their experimental results. Further, I describe the limitations this way of characterizing the phenomenon placed on the probative value of the single-molecule assay. A number of models of kinesin motility could be *conceptually* distinguished that were consistent with the HoH

activity of the other lent support to the idea that kinesin motility involves "coordinated head activity" Here, I focus principally on the single-molecule program's attempts to characterize the molecule's mechanical steps.

characterization and consistent with extant single-molecule data. However, left without adequate *empirical criteria* to distinguish between these models experimentally, researchers had to rely on indirect, theoretical argumentation to adjudicate between these merely conceptually distinct HoH models. Section 3 discusses an important 2002 study which exploited the latent experimental significance of ideas forwarded in the context of theoretical debate. This study re-drew the lines along which motility models were individuated, making *torque generation* the primary empirical criterion for individuating models of kinesin motility. This new taxonomy enabled these researchers to design a more probative single-molecule study which led them to reject HoH and forward an "inch-worm" model. Section 4 discusses the post-2002 studies that further exploited the new criterion for individuating motility models and secured consensus that kinesin walks hand-over-hand—now reconstituted as asymmetric HoH. Section 5 relates the terms of my analysis to those of (Feest 2011)'s account of how phenomena are "stabilized" and closes with a discussion of the case in light of extant philosophical models of phenomenon reconstitution.

As will be seen—and contrary to extant philosophical models—the reconstitution of kinesin motility did not occur in the context of attempting to *explain* the phenomenon, mechanistically or otherwise. Rather, it occurred entirely within the context of experimental efforts to characterize the phenomenon. More specifically, the reconstitution was driven by a recognition that individuating models of kinesin motility in terms of torque generation enhanced the probative value of the experimental program's primary investigative tool—the single-molecule motility assay. With this new taxonomy of motility models in hand, single-molecule researchers were able to use their assay to greater effect and establish a consensus that, indeed, kinesin walks hand-over-hand—now reconstituted as asymmetric hand-over-hand.

Section 1: Phenomena in Science

What are *phenomena* in science? [14] This is a vexing question addressed differently across sub-circles within the philosophy of science. Discussion of phenomena intersects, in some circles, with traditional issues of concern to philosophers of science (e.g. realism vs. anti-realism and the aim of scientific theorizing). For instance, following Pierre Duhem, constructive empiricists take the aim of scientific theorizing to be to "save the phenomena" where by "phenomena" they mean, as (Massimi 2008) puts it, "empirical manifestations of what there is" (Duhem 1908/1969; van Fraassen 1980). For philosophers of this ilk, the aim of scientific theory is to systematize phenomena under an empirically adequate (as opposed to true) theory—an aim which, it is argued, could be achieved without endorsing the reality of whatever unobservable entities the theory hypothesizes. In contrast to the postulated entities of theory, phenomena are the observable entities, processes, and events the reality of which are taken as given and which are the targets of scientific explanation. Others, (including Massimi 2008), do not attribute to phenomena the same "given" status and argue that phenomena are "constituted" in a Kantian sense of that term.[15]

Philosophers following Bogen and Woodward (1988) likewise understand phenomena as targets of explanation in science but maintain that many (if not most) of the phenomena of interest to scientists are unobservable. For instance, "the melting point of lead," "neutral currents" in particle physics, or the "chunking-effect" in human memory research are phenomena which scientists seek to explain but which cannot be observed directly. This view draws support from the fact that a large aspect of the scientific enterprise involves the development of

[14] I do not intend to develop a full answer to this question here. My analysis proceeds largely in terms of the New Mechanist view which takes phenomena to be targets of explanation in science.
[15] A sense unrelated to that in which phenomena are "reconstituted" according to the mechanists.

experimental tools and protocols which enable scientists to investigate such phenomena in spite of their unobservability. What are observable, on Bogen and Woodward's view, are data—the images, readings and values that show up on instrumentation displays and are recorded on data-sheets—which scientists use to draw inferences to the existence and character of unobservable phenomena.

The "New Mechanists" picked up this view of phenomena but moved on quickly to how phenomena are explained—specifically, advancing a mechanistic alternative to the then dominant "covering law" model of scientific explanation. In this paper, however, I am focusing on research devoted to characterizing phenomena, distinguishing it from attempts to explain them. Nonetheless, on the mechanist view—which I take as my starting point—there is a complex relation between phenomena and their explanatory mechanisms. From the point of view of one phenomenon, the organized activity of the components of the mechanism serve as explanation. But the activity of these components can themselves be phenomena. Mechanists make this point in the context of presenting multiple levels of mechanistic explanation (Figure 2.1).

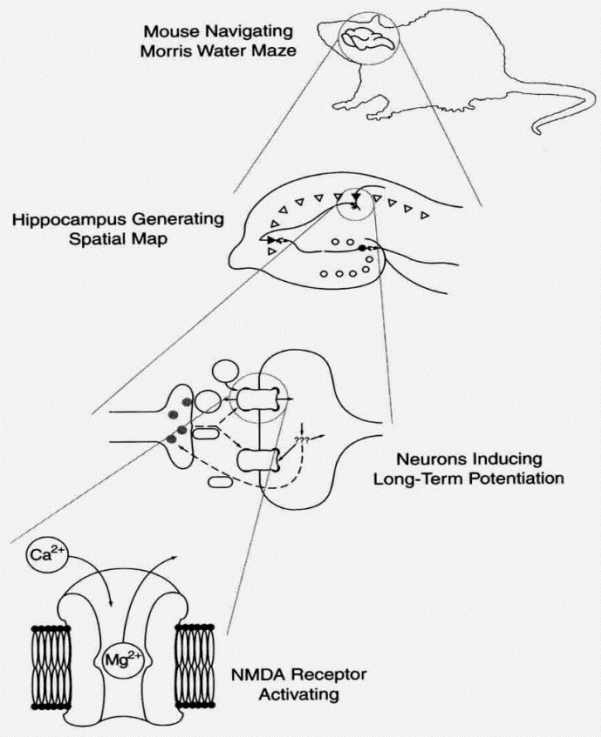

Figure 2.1: Levels of Explanation (Craver 2002)

According to the mechanists, in order to explain e.g. the behavior of mice navigating the Morris Water Maze mechanistically, we look down a level at the generation of spatial maps in the hippocampus. In order to explain the generation of spatial maps, we go further down and investigate long-term potentiation (LTP) at the neuronal level. In turn, to explain LTP, we go down to the single-molecule level to understand NMDA receptor activation. As we move down levels, we observe a shift in what is construed as the mechanism and what is construed as the phenomenon. LTP at the neuronal level, for instance, is the mechanism for the phenomenon of hippocampal spatial map generation while, from the point of view of the NMDA molecule, LTP is the phenomenon to be explained mechanistically at the single-molecule level.[16] This shifting is

[16] (Machamer, Darden and Craver 2002) give a canonical statement of this idea: "Mechanisms occur in nested hierarchies and the descriptions of mechanisms in neurobiology and molecular biology are frequently multilevel. The levels in these hierarchies should be thought of as part-whole hierarchies *with the additional restriction that*

part and parcel of the iterative process by which mechanistic explanations are produced on this view. The idea that mechanistic explanation proceeds like this—in terms of levels—is a characteristic feature of New Mechanism, distinguishing it from Ruthless Reductionism which insists that phenomena like mouse behavior in a water maze are explained directly—"in a single bound"—at the lowest molecular level (Bickle 2003).

Just as LTP is the mechanism from the point of view of one level and the phenomenon from the point of view of another, the phenomenon to be discussed here—kinesin motility—is likewise. From the point of view of explaining the phenomenon of fast axonal transport, the "walking," cargo-carrying kinesin molecule could be construed as the mechanism. A specification of the molecule's *parts* and an account of how they *operate* in an organized fashion so as to bind a cargo and "walk" along a microtubule would constitute a mechanistic explanation for the phenomenon of axonal transport. However, biologists are also interested in explaining mechanistically how the molecule manages to walk in the way that it does. Prior to being able to do so, however, researchers need a characterization of this phenomenon. Does it walk like an "inchworm?" Once it is determined that the molecules walks in *this* way rather than *that,* researchers can seek to understand the mechanical means by which it manages to walk in the characteristic way they have found it to—they can seek to explain the way that it walks mechanistically. In other words, there is 1) the way that kinesin walks and 2) the means by which it walks that way. The research discussed below using the single-molecule motility assay was aimed at characterizing kinesin's stepping pattern—the way that it walks—rather than developing mechanistic accounts specifying the means by which it walks that way. And it did so

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lower level entities, properties, and activities are components in mechanisms that produce higher level phenomena" (my emphasis).

without recourse to the explanatory electron micrographic and crystallographic work aimed at (2) that was being done in parallel.[17]

The term "phenomenon reconstitution" has not received a formal definition in the literature and I do not intend to formulate one here (although I will return in Section 5 to discuss it in more detail). That said, to indicate how I understand it for the purposes of the analysis, I first offer clarification of what phenomena are so as to be able to say how they are reconstituted. Phenomena may be understood as answering to a *what* question—*what* is the target of your explanation? One answers this question by referring to a phenomenon, for instance, "long-term potentiation" or "kinesin's characteristic stepping pattern." Once a target has been specified in this meanner, we can ask, "*by what means* does long-term potentiation occur" or "*by what means* does kinesin step in its characteristic way?" As the mechanists have it, these "means-involving" questions are answered at a "lower level" in terms of a specification of the organized parts and operations of the mechanism that generates the phenomenon—the *what.*

Phenomenon reconstitution may be characterized as an event in which there is a change with respect to the answers that researchers would give to a *what* question. For instance, we may ask "*what* are you investigating, explaining etc.?" To borrow an example from (Bechtel and Richardson 1993/2010) the answer given is, "the Mendelian trait" where "Mendelian trait" is understood in a particular way, specifically such that it is identified with a macroscopically observable phenotypic trait of an organism. Now imagine that at some point later in the history of the research program, we ask researchers the same question and they give the same answer but it is clear that what is meant by that answer is different from what was meant before. That is, we now ask our question and the researchers respond, "the Mendelian trait" but that is now

[17] In fact, as we will see, at the key moment in the story single-molecule researchers explicitly eschewed data emerging from research at the "lower" explanatory level.

identified with enzymes that are the products of single genes. In such a case we can say that the phenomenon—the *what*—was reconstituted. As we will see, something very much like this occurred in the case of the phenomenon of hand-over-hand kinesin motility. Further, as we will see, detailed scrutiny of this case enables us to understand phenomenon reconstitution in a more philosophically rigorous way. Now, on to the science.

Section 2: "Hand-Over-Hand" *circa* 1989 – 2002

By the 1980s, researchers had identified two molecules that function as motors—transforming energy into motion—myosin and dynein. (Vale et al. 1985) identified a third, kinesin, that was responsible for moving cargo such as organelles around the cell interior.

Once kinesin had been identified and named, researchers turned to characterizing its structure and behavior. (Bloom, Wagner, Pfister et al. 1988) subjected purified kinesin to centrifugation, differentiating two heavy and two light chains. They interpreted their results as showing that "bovine brain kinesin is a highly elongated, microtubule-activated ATPase comprising two subunits each of 124,000 and 64,000 daltons . . . and that the heavy chains are the ATP-binding subunits" (3409). Electron microscope studies revealed globular heads at the N-terminal end of the heavy chains, which Scholey, Heuser, Yang et al. (1989) proposed serve both to bind to the microtubule and to be the locus of ATP hydrolysis. They further hypothesized that the point of having two heads is that one remains attached to the microtubule while the other detaches and moves (Figure 2.2).

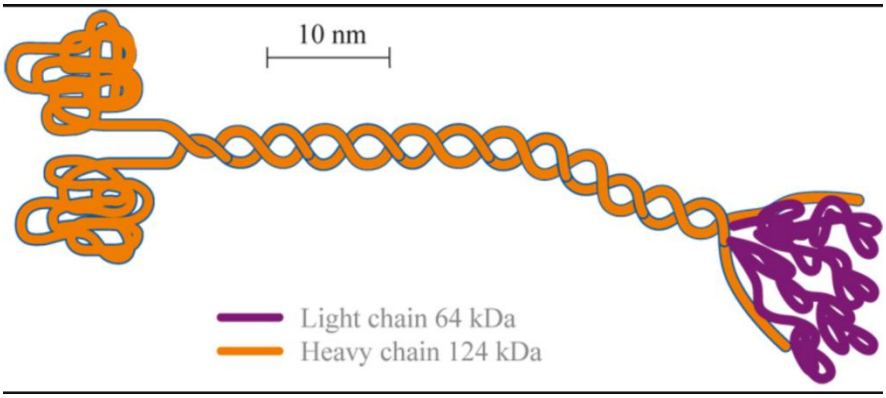

Figure 2.2: Kinesin ("Kinesin Molecule Structure" Shirinsky Vladimir P. https://eng.thesaurus.rusnano.com/wiki/article945?sphrase_id=19463 CC BY 2.0)

Howard, Hudspeth and Vale (1989) (henceforth, HH&V) reiterated this idea suggesting, on the basis of their findings using their newly developed technique for studying individual kinesin molecules, that it walks "hand-over-hand" along a microtubule. As their *single-molecule motility assay* became a central tool for investigating kinesin motility, it is worth explaining in some detail.

In order to develop an assay to investigate the motion produced by a single kinesin molecule, HH&V had first to establish that a single kinesin is capable of moving a microtubule in the first place. Their experimental design inverts how kinesin movement along microtubules may be normally understood—thinking of the microtubule as fixed and the kinesin as moving along it. Inverting this picture, these researchers immobilized kinesin molecules "heads-up" on glass cover slips in solutions containing progressively less kinesin to see how low they could go and still observe microtubules being moved along the fixed kinesin. Their hypothesis was that if a single kinesin molecule could produce movement, they should observe microtubule movement at very low kinesin concentrations. Initially finding that only when kinesin density exceeded a rather high threshold did microtubules move, these researchers distinguished two hypotheses—first, that kinesin-induced microtubule movement is a highly collaborative affair requiring a

number of kinesin molecules working in concert and, second, that kinesin denatures when adsorbed onto the coverslips and only when a sufficient number of molecules are present do a few adsorbed kinesins remain in a conformation that can support movement. Clearly, the first hypothesis, if true, would be damning for the prospects of developing an assay meant to study movement produced by a single molecule.

Optimistically assuming the latter hypothesis, HH&V pre-treated the coverslips to prevent the hypothesized denaturation. Their optimism paid off. They found that they could produce microtubule movement with one-third of the kinesin concentration required with non-treated coverslips. The clincher, however, was the character of the microtubule movement that they observed:

> Each moving microtubule rotated erratically about a roughly vertical axis through a fixed point on the surface . . . presumably as a result of thermal forces, or of torques produced when a kinesin molecule bound to different protofilaments. When its trailing end reached this nodal point, the microtubule dissociated from the surface and diffused back into solution (156).[18]

The nodal point, these researchers concluded, was a single kinesin molecule. Thus, they found that a single kinesin, immobilized on a glass cover-slip, can move a microtubule and, at the same time, developed a technique for studying this movement that would prove central to the investigation of the phenomenon of kinesin motility. More specifically, they found that a single kinesin can move a microtubule several micrometers. They reasoned that kinesin can remain attached to a microtubule by one of its heads, pushing the microtubule along as the other head moved forward, through 200 – 1000 iterations of its hydrolytic cycle. Linking this finding to the

[18] Notice the mention of "torque." The idea that HoH walking may produce torque was on the table very early on. As we will see, however, this factor was thoroughly backgrounded in subsequent discussions of experimental results taken to bear on the HoH model of kinesin motility.

fact that the molecule has two globular heads, these researchers suggested that the molecule works "hand-over-hand" with one head always remaining attached to the microtubule. However, they also suggest an alternative possibility:

> It is possible that kinesin's two globular heads work hand-over-hand, so that *one head is always bound* and prevents the microtubule from diffusing away. Alternatively, the *two heads may work independently* . . . If this is so, the time in the reaction cycle during which the kinesin heads are detached from the microtubule must be so brief, probably less than 1 ms, that the microtubule is unlikely to diffuse out of reach of the kinesin molecule (158 my emphasis).

It's important to attend closely to what "hand-over-hand" meant from the point of view of this 1989 experiment. The contrast HH&V draw between their alternatives makes clear that, as opposed to a characterization on which the heads *work independently* and, thus, on which the whole molecule (both heads) detaches from the microtubule, "hand-over-hand" has it that the kinesin heads *coordinate* their activity and that the molecule remains attached to the MT by at least one head during its walk. In other words, HoH walking consists in 1) the molecule remaining attached to the MT (*processivity*) and 2) *coordinated head activity*. These became the empirical criteria that were taken by subsequent researchers to individuate the HoH characterization as such and which informed the interpretation of experimental results for the next decade.

Over the course of the following decade, two versions of the single-molecule assay developed. 1) "MT-gliding assays," like the one already described, in which kinesin molecules are immobilized to glass cover slips and microtubule movement is observed and 2) "bead assays" in which microtubules are immobilized and kinesin-bound beads are observed to move as the kinesin attaches to and walks along the immobilized microtubule. Both "geometries" of the single-molecule assay lent support to both aspects of HH&V's HoH hypothesis.

Not all studies were immediately univocal in this respect, however. In a version of the bead assay, Block, Goldstein and Schnapp (1990) immobilized microtubules, rather than kinesin, on glass cover-slips. Coating silica beads with carrier protein and exposing them to low concentrations of kinesin, these researchers were able to observe the beads as single kinesin molecules moved them along the immobilized microtubule tracks. Using optical tweezers—which split laser beams to trap kinesins—to individually manipulate the moving beads, they found that under the forces exerted by the optical trap, the bead would detach from the microtubule after, on average, 1.4 μm and be pulled back toward the center of the trap.[19] This, they argued, provides support for the claim that, "the kinesin molecule might detach briefly from the substrate during each mechanochemical cycle" (not processive) and referred to their alternative characterization of kinesin motility as "stroke-release." (351).[20]

However, a number of influential single-molecule studies over the next 10 years strongly supported the HoH characterization over the non-processive stroke-release. In a clever variation on the MT-gliding assay, Ray et al. (1993) constructed microtubules consisting of 12, 13 or 14 protofilaments (12-mers, 13-mers, 14-mers). Protofilaments of 13-mers run parallel to the MT axis while 12 and 14-mers exhibit right- and left-handed helical organizations ("twists") respectively. Observing the movement of these microtubules induced by single immobilized

[19] The invention of optical tweezers was significant for research on kinesin motility in ways beyond those discussed here. For instance, since kinesin motility is a phenomenon occurring at the nano-scale, thermal forces are relevant. It is therefore difficult to discern what observed motion is Brownian motion and what is due to the action of the molecule. Having kinesin move cargo against the forces exerted on it by the "trap" ensures that whatever motion is observed is due to the molecule's action. This technique enabled Svoboda, Schmidt, Schnapp et al. (1993) to observe abrupt transitions of 8 nm steps, a distance that corresponds to the repeat distance between successive α-β tubulin dimers. They propose "that the two heads of a kinesin molecule walk along a single protofilament—or walk side-by-side on two adjacent protofilaments—stepping ~8 nm at a time, making one step per hydrolysis (or perhaps fewer, requiring multiple hydrolyses per step)."

[20] These researchers also suggested a model on which the molecule is always bound by at least one head but "weakly"—just strongly enough to remain attached in the face of thermal forces, but not strongly enough to remain attached when subjected to the forces of the optical trap.

kinesin molecules, the researchers found that the 12 and 14-mers rotated with the pitch and handedness predicted by the hypothesis that the kinesin molecule follows the protofilament axis. That kinesin movement is constrained in this way—that it "tracks the protofilament"—suggested that at least one head remains attached to the MT during its walk, therefore lending support to that aspect of the HoH characterization of kinesin movement.

In a version of the bead assay, Berliner et al. (1995) attached single-headed kinesin derivatives to streptavidin-coated polystyrene beads and found that, unlike intact kinesin or two-headed constructs, the single-headed molecule moved beads perpendicular with respect to the microtubule axis and failed to drive continuous unidirectional movement. This perpendicular movement suggested that the single-headed molecules lack the ability to maintain their association with a particular protofilament track, namely, another head with which to coordinate its activity. The absence of perpendicular movement suggested that the opposite is true for two-headed kinesin, lending support to the idea that the activity of the two heads is coordinated to ensure that one head remains MT-bound at all times. This, in turn assures that the molecule tracks the protofilament axis as it was found to do in the study described above.

Further support for the HoH characterization came with the introduction of fluorescent labelling in the single-molecule assay. In a version of the assay, Vale et al. (1996) directly observed the movement of individual fluorescently labeled kinesin molecules finding that the labeled two-headed kinesin travels an average distance of 600nm per encounter with a microtubule whereas single-headed constructs shows no detectable movement. This corroborated Berliner et al. (1995)'s finding discussed above, suggesting that the two heads working together is required for movement.

Hancock and Howard (1998) immobilized single-headed kinesin onto glass cover slips and found that a minimum of four to six single headed molecules are necessary to produce movement. They further showed that, even at high ATP concentration, the single-headed molecules detached from microtubules 100-fold more slowly than their two-headed counterparts "directly support[ing] a coordinated, hand-over-hand model in which the rapid detachment of one head . . . is contingent on the binding of the second head" (1395). Thus, their study demonstrated a degree of "chemical coordination" between the two heads lending biochemical substance to the idea that kinesin motility involves coordinated head activity.

Single-molecule studies such as these generated a limited consensus that kinesin walks HoH. The empirical criteria that distinguished the HoH characterization (from stroke-release) at this point in the history, are that kinesin walks processively and that it coordinated its heads' activity. The single-molecule assay provided empirical support for HoH insofar as it provided evidence that indeed kinesin is processive and that its heads' activities are coordinated. That said, a number of motility models that met the HoH empirical criteria and were consistent with extant single-molecule data were *conceptually* distinguished in the literature during this time. However, without empirical criteria by which to distinguish them *experimentally* using the single-molecule assay, it was left to single-molecule researchers to adjudicate between these models by way of indirect argumentation that appealed to data from sources external to the single-molecule program.

To illustrate, (Figure 2.3) distinguishes five stepping patterns understood to be variably consistent with the data to that time.

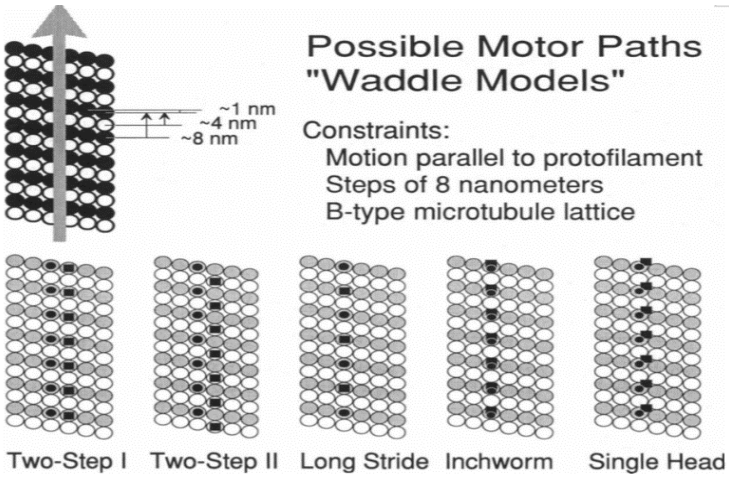

Figure 2.3: Conceptually distinguished motility models (from Block and Svoboda 1995 reprinted with permission)

Findings regarding the structure and dimensions of the molecule, the lattice structure of microtubules and the sites on tubulin heterodimers to which kinesin was understood to bind provided fodder for indirect arguments in favor of or against such conceptually distinguished models. (see Cross, 1995; Howard, 1996; Block, 1998 for reviews).[21] Microtubules consist in protofilaments arranged in cylindrical fashion.[22] Each protofilament consists of alternating tubulin (α- and β-tubulin) heterodimers. Several biochemical studies suggested that a tubulin heterodimer can bind only one kinesin head (Song and Mandelow, 1993; Walker, 1995; Tucker and Goldstein 1997). This fact, coming from outside the single-molecule program, was appealed to in adjudicating between conceptually distinct models. For instance, as we see in (Figure 2), an "inchworm model" had been distinguished prior to 2002. On this model, one head always remains in the lead with the other head trailing behind.[23] This model, however, requires each

[21]For micrographic data relevant to these indirect arguments see: (Kikkawa et al. 1994; Song et al. 1995; Harrison et al. 1993).
[22] Picture the "sheets" in Figure 2 wrapped around to form a cylinder.
[23] Though not a "hand-over-hand" model in what is perhaps the intuitive sense of the phrase, by the lights of the empirical criteria that distinguished HoH models as such (distinguished them from e.g. stroke-release models)

tubulin dimer to have two binding sites (or a single, shared binding site) so that the two heads could be brought into proximity with one another. This, argued Block and Svoboda (1995), was difficult to square with binding patterns gleaned from the aforementioned biochemical studies. They note further that such a model involves an implausibly more complicated step consisting of a "two-part cycle comprising the successive action of both heads" (237). That is, rather than each 8nm step consisting of a single head relocating to the next tubulin binding site, it would involve, first, the lead head moving and, second, the trailing head moving up from behind to keep pace.

These same researchers also argued that "long stride" seemed implausible on the grounds that it required the relatively small kinesin molecule to extend a full 16nm to move the centroid of the molecule 8nm as had been observed in their motility assays. Since this would require that the stalk connecting kinesin's heads be capable of this kind of extension, Long Stride was deemed speculatively possible at best. Cross (1995) seems to have the same worry in mind in criticizing motility models that require kinesin to stretch its heads across a protofilament, straddling it on either side, and walking along the protofilaments adjacent to it. This would be like "two-step I" only with the squares moved over one protofilament to the right. Cross says of such a model that it is "barely credible" (92).

This kind of indirect argumentation was characteristic of attempts to adjudicate between the motility models that had been conceptually distinguished in the first ten years of single-molecule research. While most researchers agreed that HoH (processivity and coordinated head activity) was the correct characterization of kinesin motility (rather than "stroke-release"), a number of models could be conceptually distinguished, all of which were consistent with HoH by the empirical criteria in terms of which this characterization was specified and all of which

"inchworm" models were a species of HoH. As we will see, it was not until the introduction of a new empirical criterion that inchworm models were adequately distinguished from HoH models along empirically tractable lines.

were consistent with extant single-molecule data. Thus, a space of merely conceptually distinct models existed to which researchers using the single-molecule motility assay had no experimental access. They were therefore left with indirect argumentation based on findings from sources external to the single-molecule experimental program.

Notably absent from most of this indirect argumentation were considerations of *torque*. This, despite the fact that HH&V had mentioned it in the very paper in which they coined the phrase "hand-over-hand." There was an exception, however. In an impressively comprehensive review, Howard (1996) did bring the idea that HoH walking produces torque into the discussion along with a number of other considerations the experimental significance of which would be exploited in a 2002 study that represented a significant challenge to the hand-over-hand consensus.

Howard (1996)'s indirect argument represents a compelling theoretical analysis. He assumes, on the basis of analogy with other known molecular motors, that kinesin has a "two-fold axis of rotational symmetry" and infers that, therefore, the heads are functionally equivalent – "they have the same hydrolysis cycles and make the same motions" (707).[24] He calls this the "equivalence hypothesis." Tracing out the consequences of this hypothesis in conjunction with extant experimental data, Howard argued that the most plausible model for kinesin motility was a "rotary model" on which the molecule's heads pass each other on the same side each step (Figure 2.4) rather than on alternating sides like the way in which our human legs move past each other as we walk.

[24] For an intuitive sense of what having a "2-fold axis of rotational symmetry" means, imagine two chairs facing each other on either side of a line and equidistant from that line. Rotating one chair 180 degrees with respect to that line will bring that chair into the precise position of its mate. Howard assumed that the relation between kinesin's two heads was the same.

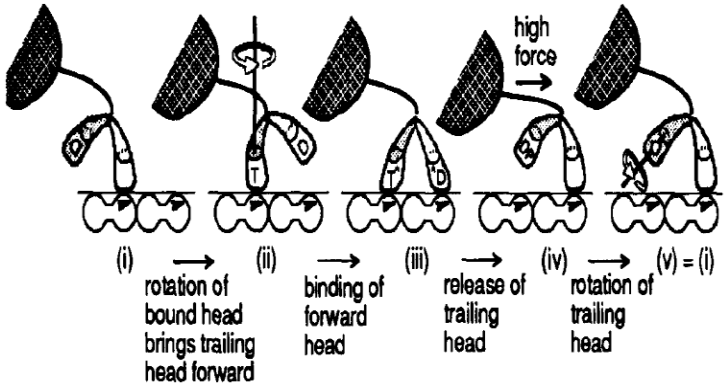

Figure 2.4: notice that state (i) is identical to state (v) (from Howard 1996 reprinted with permission)

His argument involves three key ideas the experimental significance of which was only realized later. First, taking his equivalence hypothesis in conjunction with the protofilament tracking data discussed above, Howard argues against models like the ones labeled **Two-Step** in figure 1. According to such models, the molecule switches back and forth, alternately binding adjacent protofilaments with each head. Assuming the equivalence hypothesis, a consequence of which is that the beginning of each step finds the molecule in the same 3D conformation, Howard argues that if one head (head 1), attached to a protofilament (a) were to undergo a conformational change and motion so as to bring the other head (head 2) to an adjacent protofilament (b), then the equivalent conformational change in head 2 required by the equivalence hypothesis would bring head 1 to the next protofilament over (c). This would induce a rotation in the 13-mer microtubules that was not observed in the single-molecule study discussed above. *Inter alia,* this reasoning leads Howard to his rotary model. As for the second key idea, Howard notes a "seemingly unthinkable" consequence of this model. Because of the assumed equivalence between the heads, the molecule will always rotate in the same direction and "Thus the tail (and organelle) will tend to wind up like the rubber band of a toy airplane" (724). Howard suggests

that this torsion could be accommodated by the torsional flexibility the neck was found to exhibit in an earlier study (Hunt and Howard 1993). That the neck has this torsional flexibility is the third key idea.

The experimental significance of these three ideas—1) the equivalence hypothesis, 2) that kinesin motility may produce torque which is communicated to the cargo and 3) that the kinesin neck is torsionally flexible—later came to be appreciated and exploited in a study that introduced a new empirical criterion for individuating motility models. Recall, from the late 1980s to the late 1990s, the empirical criteria that individuated HoH as such were 1) processivity and 2) coordinated head activity. From the point of view of this taxonomy, a number of motility models consistent with the HoH characterization could be conceptually distinguished that were more or less consistent with available experimental data. Adjudicating between them was left a matter of indirect argumentation using data from sources external to the single-molecule program. As we'll see, (Hua et al. 2002)'s study re-drew the taxonomic lines and, as a result, lent further probative value to the single-molecule motility assay.

Section 3: Hand-over-Hand vs. Inchworm

Hua, Chung, and Gelles (2002) inaugurated an important shift in the empirical criteria in terms of which the phenomenon of kinesin motility was investigated. As mentioned above, their study exploited ideas that had been floated in the literature in the context of indirect, theoretical argumentation. First, the design of the experiment was a modified version of (Hunt and Howard 1993)'s assay used to measure the torsional flexibility of the kinesin neck. However, rather than using native kinesin which, in that study, had been found to have a *flexible* neck, Hua and colleagues used a *stiff-necked,* two-headed biotinated kinesin derivative (K448-BIO). This

ensured that the connection between the microtubule, this molecule, and the glass cover slip on which the molecule was immobilized would be torsionally stiff, thus guaranteeing that if torque was indeed generated by the walking molecule, as Howard's model predicted, it would not be taken up by a flexible neck. Rather, it would be communicated to the cargo and generate a clearly observable 180-degree rotation of the microtubule with each step of the molecule. Their design, therefore, took the "seemingly unthinkable" consequence Howard had traced out eight years earlier and cleverly turned it into an intervention.

Further, they pointed out that whether the heads of the molecule pass each other on the same side, as in Howard's rotary model, or pass each other on alternating sides, the orientation of the molecule relative to the microtubule axis would switch as the heads alternate between being the leader and being the follower. This, in turn, would generate torque, and induce an observable microtubule rotation. In other words, the differences between the *intermediate* states of rotary models and left-right alternate stepping models were immaterial (Figure 2.5). What mattered for torque generation was that the molecule *begins* each step in the same 3D conformation only with the heads swapping between leading and following. Hua et al., dubbed these torque generating models *symmetric hand-over-hand*. By the lights of the criterion of torque generation, both Howard's rotary model and alternate left-right stepping models count as symmetric HoH models.

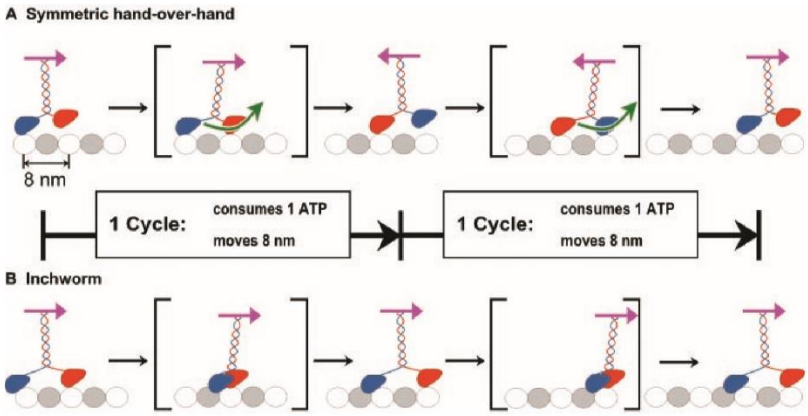

Figure 2.5: Symmetric HoH vs. Inchworm. (from Hua et al. 2002 reprinted with permission)

To appreciate the shift in criteria for individuating motility models these researchers introduced, consider the sense in which Howard's rotary model would be considered HoH prior to this study. It would count as HoH because it sees the molecule as remaining attached to the microtubule by at least one head (processivity) and that it coordinates the activity of the two heads. The same goes for alternate left-right stepping models. From the point of view of the new criterion—torque generation—both count as HoH but for very different reasons. First off, they would no longer count as HoH full stop. Rather they would be considered *symmetric* HoH to be distinguished from *asymmetric* HoH—a distinction I will discuss in more detail shortly. Further, rather than processivity or coordinated head activity serving to distinguish them as HoH (as opposed to stroke-release), they count as (symmetric) HoH because they generate torque. This, again, for the reason that both view the molecule as beginning each step in the same 3D conformation, rotating its orientation relative to the microtubule axis during its step and, thus, generating torque.

It was with respect to torque generation that the distinction between symmetric HoH and asymmetric HoH was drawn. Asymmetric HoH denies that the molecule generates torque by

denying the equivalence of the heads' steps. For asymmetric HoH, kinesin alternates between *two distinct conformations*—a different one at the beginning of each step—"in precisely such a way as to cancel the 180-degree reorientation induced by head alternation" (847).

Finally, and most importantly, after this re-drawing of the taxonomic lines, "inchworm" was no longer to be considered a merely conceptually distinct HoH model as it was by the lights of the pre-2002 empirical criteria—processivity and coordinated head activity. Now, with torque generation doing the individuative work, inchworm was distinguished from symmetric HoH along empirically tractable lines.[25]

Armed with this more probative empirical criterion by which to individuate motility models, Hua et al. (2002) developed and ran their single-molecule assay, failing to observe the microtubule rotations predicted by symmetric HoH models. They therefore rejected that characterization of the phenomenon of kinesin motility. This left two non-torque generating possibilities: 1) that the molecule walks in an asymmetric HoH fashion or 2) that it walks inchworm-style. In a way reminiscent of the indirect arguments discussed above, Hua and colleagues argued against the plausibility of asymmetric HoH. In brief, they found it implausible that the differences between 3D conformations at the start of each step could be such that they could exactly compensate for the rotation and, in turn, the torque produced by an asymmetric walk.

Interestingly, Hua et al. mention, very much in passing, a cryo-electron microscopy study which investigated kinesin at the "lower" level at which mechanistic explanations for kinesin motility were generated (Hoenger et al., 2000). This study provided some support for the idea

[25] Although it was not empirically distinct from asymmetric HoH as both inchworm and asymmetric HoH were non-torque generating. This is why, as we'll see, these researchers used indirect argumentation to argue in favor of inchworm. As we'll see later, the two became empirically distinct along "limping" lines.

that, structurally speaking, the molecule could support the kind of asymmetric walk that Hua et al. found implausible. If considerations at the explanatory level were to have played a role in the phenomenon reconstitution event that I am analyzing, this would be where they would have made their entrance—they would have offered support for asymmetric HoH. But they did not figure into the story. While that study is given a parenthetical reference, Hua et al. ignored its substance. As I said, the "inchworm episode" took place entirely within the context of an experimental program dedicated to characterizing, rather than explaining, the phenomenon of kinesin motility.[26]

So, what led these researchers to reject HoH as an appropriate characterization of the phenomenon and adopt inchworm? Note that although their rejection is experimentally motivated, they did not experiment for the purpose of gathering evidence to undermine that which had already been found in support of the HoH model. That is, they did not gather evidence to undermine the single-molecule studies that had supported the claim that the molecule is processive and that its heads coordinate their activity. Thus, they did not employ a "defeater-strategy" as in the case of "memory transfer" discussed by Colaço (2018). Rather, as described above, they recognized the experimental significance latent in certain ideas that had already been floated in the literature. They then constructed a new taxonomy using torque generation as the criterion for individuating motility models which, in turn, enabled them to design a more probative version of the single-molecule motility assay. It further enabled them to recognize an important distinction—that between *symmetric* and *asymmetric* HoH models. Their single-molecule study, they recognized, only bore directly on symmetric HoH models. Their study refuted symmetric HoH, leaving the refutation of the asymmetric model to be done by indirect

[26] Thanks to an anonymous reviewer for pressing me to show how explanatory considerations did not figure into the story.

argumentation. Thus, between their empirical results and indirect argumentation, they rejected symmetric and asymmetric HoH respectively, and defended inchworm as the most plausible characterization for the phenomenon of kinesin motility.

Section 4: Further Experimental Implications of the New Taxonomy

In section 2, we noted the role that indirect argumentation played in adjudicating between conceptually distinct models. While such arguments, in addition to the single-molecule data, led to a limited consensus, they were not decisive in adjudicating between the conceptually distinct models consistent with the HoH characterization. However, these more theoretical arguments led to ideas that had latent experimental significance. It was just a matter of unlocking it. The empirical criteria that characterized kinesin motility *circa* 1989-2002—processivity and coordinated head activity—left open an experimental dead-space seemingly inaccessible to the single-molecule assay. The key granting the single-molecule assay experimental access to the dead-space was torque generation. Turning this key generated a new taxonomy and, concomitantly, catalyzed the development of a more probative variation of the single-molecule motility assay.

The studies that emerged in the following two years took advantage of this more experimentally tractable taxonomy, re-securing a consensus that kinesin walks HoH—now reconstituted as asymmetric HoH. (Kaseda et al. 2003) tested the inchworm model's prediction that only one head is hydrolytically active. These researchers used optical tweezers in a bead assay to measure the stepping rate of kinesins mutated such that one head hydrolyzes ATP more slowly than the other. If both heads are hydrolytically active, they reasoned, their mutant molecule should show a "limp" in its stepping pattern as it walks. This is in fact what they

observed undermining the inchworm model's prediction of single-head catalysis. That same year, (Asbury et al. 2003), using optical tweezers in a bead assay, found that kinesin constructs with two identical wild-type heads also show a "limp" in their stepping, suggesting that the molecule alternates between two conformations from step to step. This supported the asymmetric HoH walking model. (Yildez et al. 2004) directly observed the movement of kinesin heads tagged with a fluorescent dye and found that each head moves 16nm per step and also that the tagged heads pause after each movement, presumably while the other untagged head moved. These findings are inconsistent with the inchworm model, which takes each head to move 8nm per ATPase cycle, and supports an asymmetric HoH model. (Higuchi et al. 2004) observed a difference in the timing of every other step in kinesins with identical mutations in the nucleotide-binding sites in each head. The limping they observed is similar to that observed by Asbury and colleagues above, but more pronounced due to the mutation.

Each of these studies exploited the reimagined taxonomy of motility models inaugurated by (Hua et al. 2002). Interestingly, it was no advancement in tool-development that enabled researchers to observe kinesin's "limping" step. The instrumentation necessary to do so—the single-molecule bead assay and optical tweezers—had been in use for over a full decade prior to its being observed. It was rather a conceptual innovation ushered in by the new taxonomy that enabled researchers to look for kinesin's limping step and appreciate its significance. Even if the limping step had been observed prior, it is not obvious that researchers would have recognized its significance, at least not in the way that it was recognized afterwards. It was in observing kinesin's limp against the backdrop of a taxonomy of motility models which included the category of asymmetric HoH that its significance for experimental work in characterizing the phenomenon of kinesin motility became apparent. Therefore, although recent philosophical

efforts to emphasize innovative tool-development in driving experimental research are to be applauded (Bickle 2016), the case of the "inch-worm episode" reminds us that conceptual innovation remains an important factor.

Conclusion: The "Reconstitution" of Hand-over-Hand Walking

Both before and after 2002, publications in this area of molecular biology regularly refer to kinesin's characteristic stepping pattern as "hand-over-hand." To a casual reader of the literature, it would not be obvious that the phenomenon of HoH walking was reconstituted within the single-molecule experimental program in the way described above. Careful philosophical analysis, however, reveals that what this term meant, as it were, changed across the "inchworm episode" in accordance with the taxonomic shifts that the episode wrought and the concomitant enhancement the single-molecule motility assay's probative value.

Before comparing my account of the inchworm episode with extant accounts of phenomenon reconstitution, let me clarify that when I say the meaning of the term changed, I mean this rather colloquially. In order to spell this out more technically, let me clarify the terms of my analysis and relate them to the terms of (Feest 2011)'s account of how phenomena are "stabilized."

To start, *a phenomenon* is an object of scientific investigation. A phenomenon is *constituted under a characterization.* A *characterization* is specified in terms of *empirical criteria*. *Empirical criteria* individuate the phenomenon along lines experimentally tractable from the point of view of a particular experimental tool. It is just insofar as a characterization is specified in terms of empirical criteria that it constitutes a *characterization.* So, since a phenomenon is constituted under a characterization and a characterization is specified in terms of

empirical criteria, episodes in which the relevant empirical criteria change constitute episodes of *phenomenon reconstitution*.

The italicized terms of technical ones which, together, express a set of tightly interrelated concepts. At the beginning of this chapter, I promised a philosophically rigorous understanding of phenomenon reconstitution. My analysis has led to the one given above in terms of this set of interrelated concepts. We can also observe that this account is helpfully general. While I argue that the "inchworm episode" represents a case of phenomenon reconstitution that was not brought about by way of explanatory considerations at the level of mechanism, my general account of phenomenon reconstitution is consistent with the fact that, sometimes, mechanistic insights can bring it about. Those insights would be ones which catalyze a change in the empirical criteria in terms of which the phenomenon is characterized. In other words, my account of phenomenon reconstitution is general enough to capture cases in which a phenomenon is reconstituted due to explanatory insights achieved at the level of mechanism and also cases, like the inchworm episode itself, in which it is not.

As we saw, the phenomenon of kinesin motility was initially constituted under a characterization specified in terms of the empirical criteria *processivity* and *coordinated head activity*. This occurred concomitantly with HH&V's development of the single-molecule motility assay. [27] It was in the very development of this tool that single-molecule kinesin motility received its initial characterization and, so, was constituted as an object of scientific investigation—a phenomenon. Upon receiving a characterization in terms of empirical criteria, alternative

[27]There is a much longer story of how the phenomenon of HoH walking was initially constituted under the empirical criteria of processivity and coordinated head activity. This is the story of how the kinesin molecule was identified in the first place and the single-molecule assay developed out of proto-versions of the protocol in which the molecule was identified. For a fascinating historical perspective see (Matlin 2020).

hypotheses regarding the character of the phenomenon could be put forward, tested, supported or refuted.

To clarify further, by "empirical criteria" I also mean those criteria which individuate characterizations of a phenomenon with respect to certain supposed features of the phenomenon that are understood or expected to give rise to characteristic patterns of data in the single-molecule assay. Feest (2011) calls such patterns of data "surface phenomena." As characterizations represent the supposed character of kinesin's movement, they represent what Feest would refer to as the "hidden phenomenon." For Feest, "stabilizing" phenomena is the process of establishing a "fit" between surface and hidden phenomena. "Empirical criteria" could be understood to supplement Feest's account. They mediate the epistemic relationship between surface and hidden phenomena. They provide the conditions that individuate models of kinesin motility (hidden phenomenon) along experimentally tractable lines, which is just to say that they indicate the kinds of data patterns (surface phenomena) expected to correspond to them.

From the point of view of the 1989-2002 empirical criteria which individuated HoH characterizations of the "hidden phenomenon"—processivity and coordinated head activity—the corresponding data patterns (surface phenomena) are of the sort generated in the single-molecule work done during the same time period and discussed in the first part of Section 2. For instance, the empirical criterion "processivity" is a supposed feature of the "hidden" phenomenon of HoH walking—one head attached to MT at all times—that is understood or expected to generate certain observable and characteristic microtubule movements in a gliding assay or bead movements in a bead assay (surface phenomena). If the molecule walks processively, researchers expect a microtubule in a gliding assay to observably (under video microscopy) glide for a prolonged period without diffusing away from the immobilized kinesin molecule.

The 1989-2002 single-molecule work represents the ingenuity of single-molecule scientists in exploring how to vary the basic design of the single-molecule assay such that it would display the data patterns expected if a single kinesin molecule walked processively and coordinated its heads. This work represents what Feest refers to as the "skill and validation" aspects of the process of "stabilizing" phenomena. This includes an "element of physical craftsmanship and . . . an element of cognitive judgment (being able to recognize that an experiment or instrument in fact works" (62). Single-molecule researchers displayed both in physically designing the assay's variations and in judging that, if the molecules walks processively and coordinates its heads, then in *this* variation of the assay *these* data should show up.

A significant number variations on the assay generated data patterns that "fit" with the HoH characterization as specified by the 1989-2002 empirical criteria. In Feest's terms, the phenomenon had been "stabilized"—researchers had "(a) empirically identif[ied] a given phenomenon and (b) gradually came to agree that the phenomenon is indeed a stable and robust feature of the world" (59). While a limited consensus had been established, however, single-molecule researchers were laboring under the limitations of the empirical criteria under which the phenomenon of single-molecule kinesin motility was initially constituted. As a result, the single-molecule assay was denied access to what I referred to above as an *experimental dead-space* consisting of merely conceptually distinct HoH models between which the single-molecule motility assay could not adjudicate.[28] Perhaps we could say, following Feest, that the

[28] For the purposes of this paper, I do not intend my term "experimental dead-space" to refer to anything other than the particular pre-2002 space of merely conceptually distinct motility models. However, I suspect that the term could refer to a general category that may be of broader utility in the philosophical analysis of scientific practice. For instance, Bogen and Woodward (1988) discuss "bubble-chamber" experiments in particle physics designed to detect "weak neutral currents." The experimental results themselves were not definitive and researchers engaged in indirect argumentation for and against the existence of weak neutral currents that deployed data and methods

phenomenon remained "unstable" to a degree proportional to the ignorance reflected in the experimental dead-space. In order to enhance the probative value of the single-molecule assay and grant it access to this dead-space, the phenomenon of HoH walking—initially constituted under a characterization specified in terms of processivity and coordinated head activity—had to be reconstituted such that torque generation became the primary empirical criteria individuating alternative characterizations of kinesin motility. In other words, in order to render the phenomenon more "stable," researchers realized that they had to start at the foundations. The very empirical criteria under which the phenomenon had been initially constituted required renovation. In short, the phenomenon needed to be reconstituted.

As I have argued, the Inchworm Episode took place entirely within the context of an experimental program dedicated to characterizing, rather than explaining, the phenomenon of kinesin motility. Though I would perhaps quibble with some of her terminology and supplement her view with the notion of "empirical criteria," the fact that the dynamics described in my presentation of the case can be well captured by Feest's account of how phenomena get stabilized (rather than explained) helps us to appreciate that the Inchworm Episode did not take place within an explanatory program. This is of particular philosophical interest as standard philosophical models have it that explanatory considerations drive phenomenon reconstitution.

(Bechtel and Richardson 1993/2010)'s model of phenomenon reconstitution, for instance, was motivated by their case study of the "Mendelian trait." Classically, the Mendelian trait was understood as a macroscopically observable phenotypic trait. Faced with the fact that patterns of phenotypic inheritance could not be explained in terms of single genes, as phenotypic traits are the products of many genes in a complex organization, researchers in the middle of the 20th

coming from outside of the bubble-chamber experimental program. This may constitute another instance of an experimental dead-space. See Bogen and Woodward (1989) pgs. 228-230.

century abandoned the phenotypic trait as the central Mendelian unit in favor of a unit at a lower level of mechanistic analysis, the *enzyme.* Thus, the explanandum phenomenon to be accounted for in terms of single genes was reconstituted, shifting it down from the phenotypic trait to the enzyme, in the effort to develop mechanistic accounts of gene action.

(Craver 2007) discusses a further way in which phenomena can be reconstituted in the context of seeking mechanistic explanations. According to Craver, phenomena can be reconstituted in the wake of researchers recognizing that they have committed one of two errors – the "lumping error" or the "splitting error." Both errors require inquiry into the phenomenon to have developed to a point at which researchers have both a characterization of the phenomenon and putative mechanistic explanations on the table. Scientists observe they have committed the splitting error when they recognize that they have erroneously thought that some phenomena of interest are due to two or more distinct types of mechanisms when, in fact, they are due to mechanisms of the same type. They may then reconstitute the phenomena such that where once they thought of them as two distinct phenomena underpinned by two distinct types of mechanisms, they now understand them as one phenomenon underwritten by a single mechanism-type. The lumping error, on the other hand, occurs when a particular phenomenon is thought to be generated by a single mechanism while, in fact, two distinct mechanisms underwrite the phenomenon. In light of recognizing this error, scientists may reconstitute the phenomenon, considering it now as two distinct phenomena.

(Kronfeldner 2015)'s model differs from both of the above. She describes how phenomenon reconstitution can result not only as a result of researchers gaining insight at the level of mechanism, but also by researchers moving up to a level of greater abstraction. To illustrate, a researcher interested in explaining a particular phenotypic trait of a particular

person—their height, say—will be unable to do so as it is widely recognized that such traits are the result of complex interactions between an individual's genetic inheritance and their ontogenetic environment. This does not mean, however, that genes do not explain. By moving up to an explanandum phenomenon at a greater level of abstraction, e.g. average differences between the heights of males and females in a population, researchers can appeal for explanation to differences in genotype, ignoring the complexity introduced by gene-environment interactions. In this way, researchers can hold fast to a particular "causal factor" in terms of which they wish to pitch their explanations and constitute the phenomena to be explained accordingly.

All three models have it that phenomenon reconstitution is driven by explanatory considerations. The research on kinesin motility discussed throughout this paper, however, involves experimental work dedicated solely to characterizing (stabilizing) the phenomenon of kinesin movement. Developing mechanistic explanations of kinesin movement (not discussed) involves researchers determining how the energy released from ATP-hydrolysis occurring in the molecule's nucleotide binding sites results in characteristic structural changes throughout the molecule. Mechanistic explanation asks after the role played (if any) by thermal forces in bringing the heads forward in their stepping pattern. It attempts to determine whether elastic tension on the neck linker generated as the molecule stretches during its walk provides energy—in addition to that provided by ATP-hydrolysis—that may or may not be necessary for walking. These (and further issues) are, of course, important for developing mechanistic explanations for kinesin motility—for answering the question of *by what means* kinesin manages to walk in the way it does. But considerations at this explanatory level did not, as we saw, figure into the reconstitution story. Again, it took place entirely within the context of experimental efforts to

characterize the phenomenon—to characterize the way kinesin walks, not the means by which it manages to walk that way.

Colaço (2020) notes "there is a lacuna in the literature regarding how researchers determine whether their characterization of a target phenomenon is appropriate for their aims" (1). Colaço helps illuminate this lacuna, using a case study to show the way in which our understanding of phenomena should be revised that do not depend on explanation. My analysis of the Inchworm Episode sheds further light. In order to experimentally adjudicate between alternative characterizations of kinesin motility, single-molecule researchers sought *empirical* criteria by which to individuate them—criteria that distinguished them along lines that were testable from the point of view of the single-molecule motility assay. It was determined that individuating characterizations of kinesin motility by appeal to torque generation rather than merely processivity and coordinated head activity, enabled access to what was antecedently an experimental dead-space consisting of merely conceptually distinct motility models. The new taxonomy rendered that space experimentally accessible to the single-molecule motility assay. Thus, the Inchworm Episode illustrates how researchers can recharacterize—or, better, reconstitute—phenomena to the end of enhancing the probative value of their experimental tools.

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Part 2: Explanation

Chapter 3: Active Biological Mechanisms: Transforming Energy into Motion in Molecular Motors

> In recent years, interest in biological motion has blossomed because of the realization that much of cell behavior and architecture depends on the directed transport of macromolecules, membranes, or chromosomes within the cytoplasm. Indeed, modern microscopy has transformed our view of the cell interior from a relatively static environment to one that is churning with moving components, not unlike the bustling traffic in a metropolitan city. (Vale & Milligan, 2000)

Section 1: Introduction

According to the New Mechanist philosophy of science, explaining a phenomenon involves specifying the organized entities composing the mechanism responsible for it. Importantly, the entities in a mechanism are *active* and a specification of the activities in which they engage is necessary for understanding how the mechanism undergoes characteristic changes that produce the phenomenon it explains. As Machamer, Darden and Craver (2000) (henceforth, MDC) write:

> [I]t is . . . impoverished to describe mechanisms solely in terms of entities, properties, interactions, inputs-outputs, and state change over time. Mechanisms do things. They are active and so ought to be described in terms of the activities of their entities, not merely in terms of changes in their properties. (p.5)

Drawing from a specification of the mechanism responsible for the action potential in neurons, MDC's list of activities includes, to name only a few, "fitting, turning, opening, colliding, bending and pushing . . . "(Machamer, Darden, & Craver, 2000). Other mechanists agree. Consulting biochemistry textbooks, Illari and Williamson (2013) list "trigger, binding,

phosphorylates, modifying, wrapping . . . unwinding, supercoiling . . . and stabilizing" as terms denoting activities.

There is some debate, however, over whether activities, as an ontological category, are required or even helpful in understanding mechanisms. Some, like Cartwright (1999), argue against including activities in our ontology and favor *capacities*, "The knowledge we have of the capacity of a feature is not knowledge of what things with that feature do [activities] but rather knowledge of the *nature* of the feature." Others, like Machamer (2004), insist on activities, "One can't specify a . . . capacity without having some way to identify what the capacity does when it is actualized or exercised. However, being able to recognize what a capacity does when actualized or the activity that constitutes it presupposes having the concept of activity."

Our focus here, however, is not on the metaphysical issue of whether and how to incorporate activities into the ontology of science but with the question of how to *explain* activities. For those concerned with ontology, the fact that scientists assert that the alpha helix in a sodium channel *rotates* in response to the spreading depolarization of the axon argues that "rotatings" need ultimately to show up in a "descriptive ontology of science."[29] This leaves the question: *by what means* does this rotation occur? More generally, by what means do the entities in a mechanism engage in their activities? From this point of view, merely attributing to activities a positive ontological status is unilluminating. As Winning and Bechtel (2018) write, "The activity of a mechanistic component is *what* the component actually does; it is not the *why.* For this reason, we contend that what is needed is an account . . . of whatever it is about mechanisms . . . in virtue of which activity is brought about." We follow them here in thinking that simply countenancing activities in our ontology does not help us understand activities in mechanisms.

[29]We borrow this phrase from Illari and Williamson (2013).

To do so is to merely re-state, in a round-about (and fruitlessly reifying) way, the fact that mechanisms are active. What is important is to give an account of the means by which they are.

We cannot make progress in addressing this question by engaging in *a priori* speculation. Just as the New Mechanists in philosophy of science developed their accounts of mechanisms by examining actual science (Bechtel & Richardson, 1993/2010; Bechtel & Abrahamsen, 2005; Machamer et al., 2000; Craver & Darden, 2013; Glennan & Illari, 2018), we need to proceed by examining instances in which scientists have had success in addressing this question. Like the New Mechanists, we focus on examples from biology. Fortunately, there are a number of examples in recent biology in which researchers have addressed just this question—they have not only garnered evidence for the occurrence of certain types of activities but have also offered explanations of these activities. To do so they have had to go beyond decomposing the mechanism into constituent entities performing their activities, since there is little gain, when the question is to explain how activities are active, in explaining one activity in terms of others. In order to avoid a regress of activities—to avoid the conclusion that mechanistic explanation explains an activity in terms of others—one needs an account of the means by which mechanisms are active that does not itself appeal to the category of activities.

A key component of these accounts of biological activities is identifying the source of Gibbs free energy that is utilized in the activity. Although a focus on the source of free energy has notably been lacking from the various characterizations of mechanisms and mechanistic explanation, a basic principle from physics that is honored in biology is that no work can be performed without a source of free energy. The challenge in explaining activities is twofold—to identify the source of free-energy and to understand how that free-energy is converted into a specific activity. At a generic level, as argued by Winning and Bechtel (2018), the latter depends

on how the constituent parts or entities of a mechanism constrain the flow of free energy into the performance of a particular activity. This is familiar in the case of human-made machines such as a car: the free energy released in the combustion of gasoline is constrained to generate mechanical motion by exerting force on a piston which is then passed, through the driveshaft, to the wheels. In this instance, heat is the intermediary, but in living organisms heat is recognized as a waste-product—its diffusion is not constrained to produce work. How, then, in biological mechanisms, is free energy constrained to produce work? Understanding that will provide a foundation for understanding how mechanisms in biological organisms are active.[30]

To address the question of how free energy is transformed into activity in biological mechanisms, we focus on a particular class of cellular mechanisms, molecular motors that convert free energy, in the form of ATP, into the exertion of force, either on objects external to the cell or other components of the cell. For this paper we limit ourselves to two molecular motors, myosin II (hereafter, myosin), which pulls itself along actin fibrils, resulting in contraction of muscles, and kinesin-1 (hereafter, kinesin), which pulls cargo toward the periphery of a cell along microtubules.[31] In many respects, research on these motors follows the familiar

[30] The relation of entities or activities to sources of free energy parallels Klein's (2018) characterization of the relation between mechanisms and resources. On Klein's account, resources may either be available or not, are not individually important, potentially interact promiscuously with the parts of a mechanism, but are nonetheless essential for the functioning of a mechanism. Klein applies his account of a resource to gasoline, the form of free energy for a car, but these features are true of sources of free energy in general. Winning and Bechtel's analysis shows why free energy is a needed resource and how it figures in the understanding of the mechanism—the activities performed by the mechanism result from free energy being constrained by the components of a mechanism. This points, though, to an important difference: for Klein, resources are the patient of action, whereas on Winning and Bechtel's account, free energy is the source of activity in a mechanism.

[31] Myosins and kinesins constitute a natural category since both are P-Loop ATPases that are very similar to G-proteins (Kull, Vale, & Fletterick, 1998). There are several other classes of molecular motors that hydrolyze ATP to generate motion, such as dyneins. Neither *myosin* nor *kinesin* designates a single protein. There are, in fact, at least 35 different classes of myosins, 13 of which have members occurring in humans. Although it was the first discovered myosin, muscle myosin is now designated myosin II. After the discovery of the first kinesin, 14 different classes of kinesins have been identified. The originally discovered kinesin, sometimes referred to as *conventional kinesin*, is a member of the kinesin-1 family. We will focus on myosin II and conventional kinesin, appealing to research on other myosins and kinesins as it contributed to the understanding of myosin II and conventional kinesin.

picture of mechanistic research: scientists identified phenomena of interest and then decomposed the responsible mechanism to show how it generates the phenomenon. But there are important differences between these endeavors and those that have been the focus of the New Mechanists. First, a key element was describing how free energy, in the form of ATP, figures in the operation of the motor. Motors are ATPases—enzymes that break the bond between the third (γ) phosphate group and the rest of the molecule, releasing free energy. The challenge was to link the stages of the molecules' hydrolytic activity with the stages of their mechanical movements. We develop this in section 2. However, as we discuss in section 3, researchers' explanatory efforts did not stop there. Rather, taking these activities as phenomena to be explained, they developed accounts of how the free energy released by ATP hydrolysis in the constrained environment of the molecules results in their characteristic activities—stepping along actin filaments, in the case of myosin, and along microtubules for kinesin. Importantly, these accounts are not given in terms of further entities and activities in a mechanism at a lower explanatory level. Rather, they are given in terms of *constraints* and *energetics*. Section 4 fleshes out the account and suggests that the lesson we extract for the case of molecular motors research generalizes to mechanisms other than molecular motors. Biological mechanisms and their components are indeed active, but this activity is explained in terms of the constrained release of free energy. We conclude that while the motors we discuss constitute "bottom-out entities" (the term was introduced by MDC) for mechanistic explanation in biology, analysis of mechanisms at higher levels can benefit from construal in terms of energetics and constraints. Identifying how free energy is constrained, however, is not, strictly speaking, necessary as higher-level mechanistic explanations can simply appeal to entities and activities. Recognizing that one can further explicate activities in terms of constrained release of free energy dispels the metaphysical mystery that results when activities

are construed as ontologically fundamental. Section 5 summarizes what we claim to have accomplished.

Section 2: Characterizing the Role of Free Energy in Molecular Motor Movement

In this section, we describe how researchers developed accounts of the mechanisms responsible for muscle contraction and axonal transport. In both cases, the relevant mechanisms are molecular motors—myosin and kinesin respectively. The research involved identifying the motors, characterizing the movements—activities—of their parts, and mapping the stages of these movements onto stages of the motors' hydrolytic cycles. At this stage, researchers had identified the mechanisms for the phenomena of interest and specified them in terms of their entities and activities. But, as we will see in section 3, their explanatory efforts did not stop there. Rather, researchers developed accounts of the means by which these mechanisms engaged in their activities according to which the release of free energy due to hydrolysis in the molecules' constrained environment results in their characteristic stepping activities. Importantly, this further account was not given in terms of still more entities and activities but rather in terms of constraints and energetics.

2.1: Identifying ATP hydrolysis in the operation of the myosin motor

Leeuwenhoek initiated the microscopic examination of muscle, but it was with the much-improved microscopes and advent of staining techniques in the mid-19th century that researchers advanced the now classic descriptions of anisotropic (A) and isotropic (I) bands on muscle fibers and described how A bands shortened while I bands lengthened when muscles contracted. During

the same period Kühne (1864) isolated from muscle-press juice when the muscle was in the rigor state what he took to be a single viscous molecule he called *myosin*. Subsequent researchers attributed myosin to normally functioning muscles and proposed that muscle contraction resulted from changes in myosin. In the wake of the discovery of adenosine triphosphate (ATP) and characterization of it as an energy source for biological activities, Engelhardt and Ljubimowa (1939) established that myosin functioned as an ATPase. In research conducted during World War II but only published in a widely accessible venue afterwards, Szent-Györgyi (1945) showed that what had been taken to be one molecule actually consisted of two proteins, myosin and actin. Both actin and myosin constitute filaments, but only myosin functions as an ATPase (for detailed historical accounts, see Needham, 1971; A. F. Huxley, 1977; Rall, 2014).

Before researchers could establish how ATP hydrolysis figured in muscle contraction, they needed to discover how myosin moved with respect to actin. A pair of papers published back-to-back in 1954, one by A. F. Huxley and Niedergerke (1954) using interference microscopy and one by H. E. Huxley and Hanson (1954) using X-ray crystallography and electron microscopy, revealed a critical feature: crossbridges between thin (actin) and thick (myosin) filaments. To characterize the activity of these crossbridges, researchers had to procure multiple images that showed the cross-bridges in different states of muscle contraction. From such evidence, H. E. Huxley (1958, 1969) articulated the crossbridge hypothesis (shown in Figure 1a) according to which a bridge that projects from myosin to actin goes through a cycle of stages in which it detaches from actin (1), moves (2), reattaches to actin (3), and exerts force in a "powerstroke" to pull itself along the actin filament (4).[32]

[32] See Hitchcock-DeGregori and Irving (2014) for a detailed analysis of Hugh Huxley's contributions.

Independently, biochemists Lymn and Taylor (1971) added a radioactive tracer into the third phosphate group of ATP and followed the process of ATP hydrolysis by myosin. This revealed both rapid hydrolysis of ATP to ADP and Pi and a much slower release of Pi unless myosin is bound to actin. They mapped these steps onto the steps in the movement of the crossbridge in what came to be known as the Lymn-Taylor cycle (Figure 1b). In the first step myosin binds ATP and detaches from actin (1). As ATP is hydrolyzed the crossbridge returns to a right angle (2); it then binds to a new locus on myosin (3). Myosin remains bound to the reaction products (Pr), ADP and Pi, until step 4, at which their release corresponds to a movement of the crossbridge.

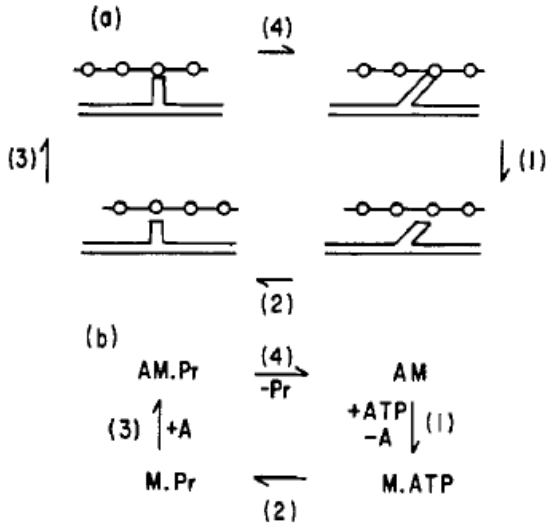

Figure 3.1: The Lymn-Taylor cycle. From Lymn and Taylor (1971)

Integrating the crossbridge hypothesis and the Lymn-Taylor cycle yielded a coherent description of the relation of ATP hydrolysis to myosin movement that, in its basics, has been adopted in subsequent research and significantly elaborated on. The key force-applying step that causes the myosin filaments to pull themselves along actin filaments is known as the *powerstroke* (step 4 in

Figure 1). A key feature of the account is that while hydrolysis of ATP provides the energy for the powerstroke, ATP is not directly involved in that step. The powerstroke occurs with the release of ADP and Pi that had resulted from hydrolysis in step 2. At the time of hydrolysis, the crossbridge is not attached to actin. Only after myosin rebinds to actin and successively expels Pi and ADP does the powerstroke occur which causes the crossbridge to change conformation and move approximately 10 nm with respect to actin. Myosin remains tightly bound to actin (this is known as the rigor state as it was assumed to correspond to the state assumed in rigor mortis) until it binds to another ATP molecule, whereupon it detaches from actin.

2.2: Identifying ATP hydrolysis in the operation of the kinesin motor

Kinesin was only discovered in 1985 based on two lines of research. The first identified microtubules as fibrils within axons in electron micrograph studies (De Robertis & Franchi, 1953; Palay, 1956). In subsequent research these were shown to consist of tubulin molecules that self-organize in a polar fashion, with what is identified as the +-end pointing away from the cell center. The second, relying on radioactive tracers, revealed the transport of proteins and larger structures along axons (see Grafstein & Forman, 1980, for a review). Combining techniques of video and differential interference contrast microscopy, Allen and his collaborators (Allen, Allen, & Travis, 1981; Allen, Metuzals, Tasaki, Brady, & Gilbert, 1982) showed that this transport occurred along microtubules in both directions, with the cargo sometimes falling off and remaining motionless until another microtubule "came along to provide a substrate or carrier for the movement." Adams (1982) and Lasek and Brady (1984, 1985) showed that movement along microtubules depended on ATP. To identify the responsible ATPase protein, Vale, Reese, and Sheetz (1985) centrifuged squid axoplasm, suspended the resulting particle in ATP-containing

buffer, and added the product to microtubules placed on a glass coverslip. Observing movement of carboxylated latex beads along the coverslip and noting that the molecular weight of this particle was distinct from both myosin and dynein (the two molecular motors then known), they concluded that it was a novel force-generating protein and named it *kinesin* (from *kinein*, Greek for "to move").

Subsequent work focused both on characterizing the structure of kinesin as consisting of two globular heads and tail structures which bound to cargo and showing that the heads hydrolyzed ATP only when bound to microtubules (Scholey, Heuser, Yang, & Goldstein, 1989). Howard, Hudspeth, and Vale (1989) developed an important technique, the single-molecule motility assay, through which they showed that a single kinesin molecule can produce movement and suggested that it moves along the microtubule by "coordinating" the activity of its heads such that one head releases at a time and moves past the other thus, walking in a "hand-over-hand" fashion (Bollhagen 2021). Schnapp, Crise, Sheetz, Reese, and Khan (1990) proposed that this coordination was achieved by the binding of one head to ATP. This causes a conformational change in the other head, allowing it to bind to the microtubule and release nucleotide. The hydrolysis cycle thus "coordinates" the activity of the heads to ensure that one remains attached to the microtubule at all times (figure 2). Research in the following decade demonstrated that kinesin heads moved forward in discrete steps that correspond to the 8 nm repeats of tubulin (Svoboda, Schmidt, Schnapp, & Block, 1993) and that just one ATP was hydrolyzed per step (Schnitzer & Block, 1997; Hua, Young, Fleming, & Gelles, 1997; Coy, Wagenbach, & Howard, 1999).

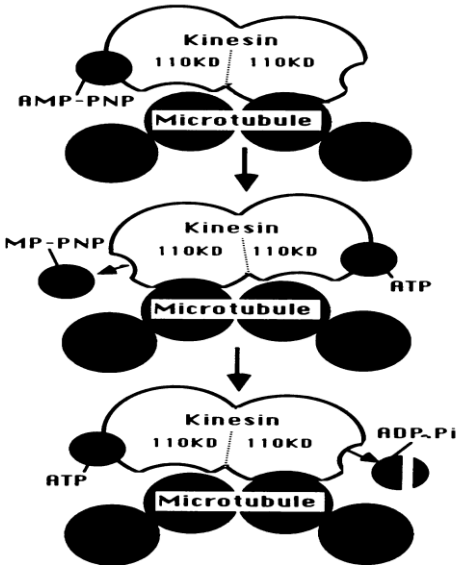

Figure 3.2: Schnapp et al., 1990.

Further developing the account of how the steps of ATP hydrolysis map onto the movement of kinesin's two heads required use of more traditional biochemical techniques. Hackney (1994) found that when two-headed kinesin molecules with both heads bound to ADP associate with microtubules in the absence of ATP, only 50% of the ADP is released, whereas in the presence of ATP 100% of the ADP is released. Further, 100% of ADP is released when a single headed kinesin construct binds to a microtubule in the absence of ATP. These findings suggested that when one head binds to a microtubule, it releases its ADP but that the other head is prevented from binding to the microtubule until the first head binds another ATP. Hancock and Howard (1999) further elaborated on the scheme of coordination by comparing the normal two-headed kinesin with a heterodimeric one-headed kinesin, showing that without the second head the kinesin detached an order of magnitude slower. From this they claimed that in the normal kinesin an internal strain between the heads serves to coordinate them, with the head bound to

ADP being affected by the activity of the other head in expelling ADP, binding ATP, and hydrolyzing it (figure 3).

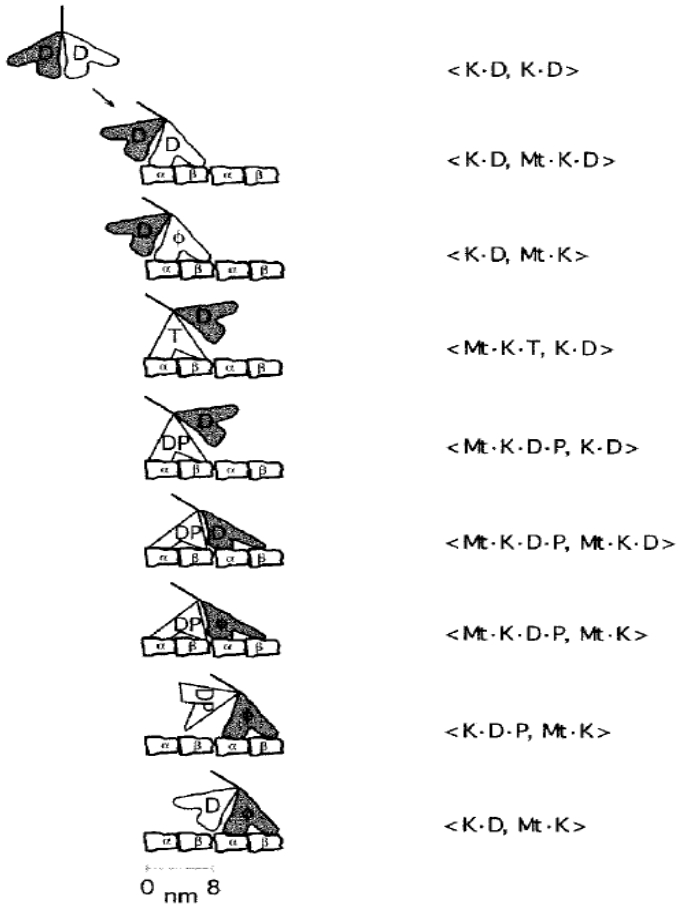

Figure 3.3: Hancock and Howard (1999)'s kinesin chemomechanical cycle.

2.3 Explaining Motor Movement in Terms of the Activities of ATP Hydrolysis

In this section, we have discussed how researchers developed mechanistic explanations for the phenomena of muscle contraction and intracellular transport. Researchers identified the responsible motors—myosin and kinesin, respectively—and decomposed the motors into entities and activities. The molecules' heads (entities) hydrolyze ATP (activities) which leads to the

stepping (activity) of the heads. This is expressed economically in the diagrams which map the stages of the heads' hydrolytic cycles onto the stages of their mechanical steps. These mappings constitute a specification of the mechanisms for muscle contraction and intracellular transport in terms of the relevant entities and activities.

By certain philosophical standards, this is a remarkably complete explanation. Not only have the relevant entities and activities been identified but, further, these mappings establish relations of counterfactual dependence between stages of hydrolysis and stages of mechanical motion. Consider, for instance, step 1 of the Lymn-Taylor cycle depicted in Figure 1. The diagram supports the following claims: 1) If the myosin head binds ATP (activity), the molecule releases the actin filament (activity). 2) If it does not bind ATP at that stage, the motor does not release from the actin filament. Likewise, the occurrence of each other stage in myosin's mechanical movement counterfactually depends upon the occurrence of the corresponding stage in the molecule's hydrolytic cycle. Arguably, this licenses a causal claim—the relevant stages in the molecule's hydrolytic activities *cause* the corresponding stages in their mechanical steps.

One might also think about the achievement these mappings represent in the following way. The representations of the stages of the molecules' mechanical steps (without the steps of ATP hydrolysis mapped onto it) constitute descriptions of the mechanisms merely in terms of state-transitions. As it stands, this is explanatorily inadequate by standard mechanist lights, "we think state transitions have to be more completely described in terms of the activities of the entities and how those activities produce changes that constitute the next stage (Machamer, Darden and Craver 2000, p. 5). The concern here is that mechanisms are *productive* and a representation of a mechanism in terms of mere state-transitions fails to capture the productivity essential to understanding how a mechanism generates a phenomenon. By superimposing the

stages of ATP hydrolysis onto the representations of the molecule's mechanical steps, we can see that the stages in the molecules' hydrolytic activities are the activities that advance the motors through their state transitions. Arguably, then, the mapping captures just that element of productivity that the standard approach to mechanistic explanation requires. Who could ask for anything more? Well, as we will see in the next section, biologists themselves.

Section 3: Explaining the Activities of Molecular Motor Movement

The research described in the previous section showed how the source of free energy, ATP, is coupled to the stepping activity of the myosin and kinesin motors by mapping stages in ATP binding, hydrolysis, and release of the products to stages of myosin's and kinesin's mechanical stepping. By standard mechanist lights, these constitute mechanistic explanations for the phenomena of muscle contraction and intracellular transport. As we will see in this section, however, researchers went further, and sought accounts of the means by which the molecules' hydrolytic activity causes the molecules to step. With its adherence to a fundamental dualism of entities and activities, the standard account of mechanistic explanation predicts that these accounts will be given in terms of a mechanistic explanation at a lower explanatory level that likewise specifies further entities and activities. This was not the case, however. Rather, researchers came to understand the molecules as themselves constituting characteristic sets of constraints on the free energy released in the course of ATP hydrolysis. In the constrained environment of the molecules, the chemical energy stored in ATP is transformed into mechanical energy to realize the stepping activities of the motors.

3.1: Explaining the Activity of Myosin

The first representation of the structure of the ATP binding site in myosin was created by Rayment et al. (1993) based on protein crystallography[33] of chicken skeletal muscle (Figure 4). Since this could be fitted to EM reconstructions of "decorated actin" produced by incubating actin filaments with isolated crossbridges without ATP, the researchers interpreted the image as showing the rigor state after ADP and Pi had been expelled and with myosin attached to actin (upper right corner in figure 1A and 1B). What this image showed was that the ATP binding site (labeled Nucleotide binding site) is at the opposite end of a β-sheet from the actin binding region, which is situated at the end of a cleft between the Lower 50K and the Upper 50K domains. Significantly, these sites are separated by about 40-50 Å. As a result, researchers concluded that the effects of the respective binding to actin or nucleotides must be communicated mechanically to the other site by physical changes in the β-sheet. The images also revealed a long helical tail, consisting of an α helix, that has the appearance of a lever arm. This tail is rigidly attached to the converter domain, suggesting that hydrolysis of ATP results in movement of the converter and the attached lever arm.

[33] The crystal structure for actin has been generated a few years earlier by Holmes, Popp, Gebhard, and Kabsch (1990). Actin was generally viewed as a passive component in muscle contraction, although it is now recognized to play crucial regulatory roles in the behavior of myosin.

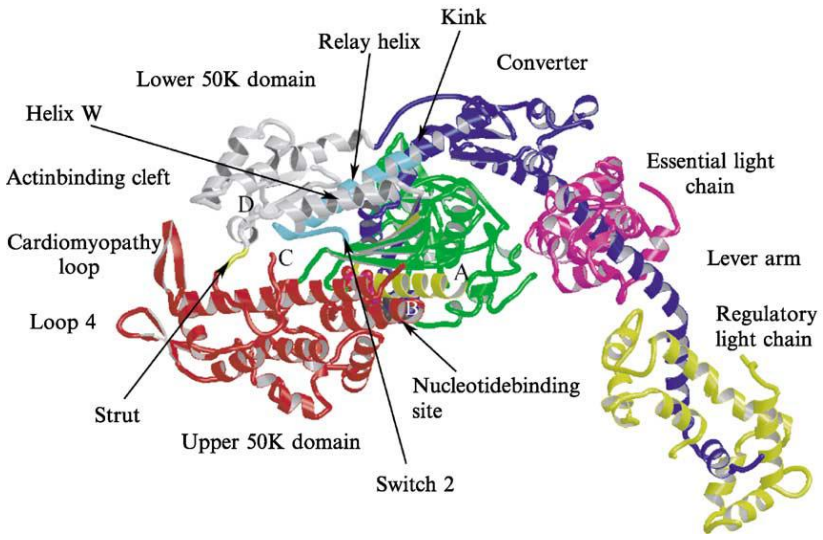

Figure 3.4: Ribbon diagram of myosin motor domain in post-rigor state based on Rayment et al. (1993). Nucleotide binding site is shown in yellow. Actin binds in the cleft between the Upper 50K domain and the Lower 50K domain. The lever arm is in the post-powerstroke or *down* position. From Geeves et al. (2005).

Individual crystal structure images are static and so do not reveal movements. To figure out the specific physical movement generated by hydrolysis that then resulted in movement along actin, researchers needed to compare this image with ones generated in other states. A few years later, several researchers (Smith & Rayment, 1995; Smith & Rayment, 1996; Fisher et al., 1995) generated crystal structures of myosin bound to molecules that bind as ATP does but do not undergo hydrolysis. These crystal structures were interpreted as presenting the pre-powerstroke whereas the first image represented the post-powerstroke state. Comparing the images revealed that the converter and lever arm had shifted by 60-70° in the powerstroke. This angular difference was proposed to correspond to the 10 nm movement imposed on actin (see Holmes, 1996; 1997, for reviews). This provided an account of how the force generated in hydrolysis was transmitted to other parts of the myosin molecule.

Crystal structure images also revealed the structure of the ATP binding site—it was seen to involve a P-loop motif and switch 1 and 2 segments, very similar to those found in G-proteins.

(Only switch 2 is shown in Figure 3.4; switch 1 is in the region between the areas marked A and B.) The comparison of the pre-powerstroke and rigor-like state shows that switch 2 moves 5 Å: it starts in the closed state in contact with the γ-phosphate (Pi) and enters the open state when it moves away. As in G-proteins, the closing of switch 2 involves the formation of an amide hydrogen bond between a glycine and the γ-phosphate of the nucleotide as part of the mechanism of hydrolysis. The formation of this bond also forces the Lower 50K domain to rotate. This in turn forces movement in the attached relay helix (so named since it represents the communication pathway between the nucleotide binding site and the converter domain). Since the relay helix is forced up against a β-sheet, the attempted movement generates a kink that rotates the converter and lever arm 70º (Smith & Rayment, 1996; Holmes, 2008). This tension stores the energy that will be released in the powerstroke.

Research over the subsequent two decades have led to important revisions and additions to this account,[34] but they only reinforce the general picture that the chemical energy released in ATP hydrolysis is transformed into physical movement in the ATP binding site, which is then communicated to other parts of the myosin motor, changing its shape and hence its action on actin. This is illustrated in a recent model developed to accommodate evidence, based on FRET (fluorescence resonance energy transfer), that shows that ADP and Pi are released in different steps with both occurring after the powerstroke (Muretta et al., 2015; Muretta, Petersen, &

[34] For example, images by Coureux et al. (2003), Holmes, Schroder, Sweeney, and Houdusse (2004), and Holmes, Angert, Jon Kull, Jahn, and Schröder (2003) showed that when myosin is the rigor state, bound to actin without the nucleotide, the cleft between the Upper 50K and Lower 50K domains is closed. Drawing on these, Coureux, Sweeney, and Houdusse (2004) advanced an interpretation according to which binding to actin rotated the Lower 50 K domain and resulted in the opening of the nucleotide binding site. When a new ATP molecule is bound into the nucleotide site, the rotation of the Lower 50 K domain is reversed, reopening the actin binding site. Binding a new ATP also alters the conformation of the P-loop and switches 1 and 2 so as to favor ATP hydrolysis. This further supported the interpretation that the opening of Switch 2 rotates the β-sheet and creates a kink in the relay helix that moves the converter, creating mechanical stress which is released in the power stroke and causes the lever arm to rotate back.

Thomas, 2013). To fit the currently available data, Houdusse and Sweeney (2016) have advanced the scheme shown in Figure 3.5. On their scheme, the powerstroke through which the myosin exerts force to pull itself along actin occurs after ATP hydrolysis and before the release of ADP and Pi. During the powerstroke the force built up in the abnormal position of the lever arm is released, and the lever arm moves 90°, back to its default position. Since the myosin is bound to actin at this time, the effect is to pull the rest of the myosin filament along the actin filament. Once the ADP and Pi are expelled, myosin detaches from actin and prepares for another powerstroke by binding a new ATP. This conformation change at the binding site results in re-cocking the lever arm. Since myosin is not attached to actin, the myosin head moves freely and is positioned further along the actin. Little free energy is released at this step. With hydrolysis and the subsequent binding of myosin at the new position on actin, force is built up, ready to be released with a new powerstroke. Thus, the free energy released in ATP hydrolysis is temporarily constrained to maintain the lever arm in its position until, in the next powerstroke, it is released.

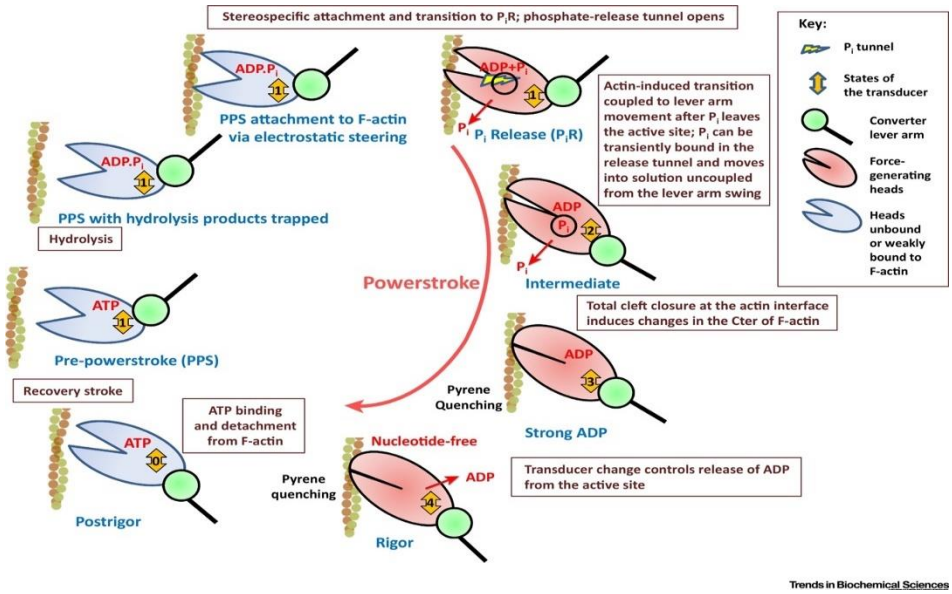

Figure 3.5: How ATP hydrolysis generates forces that are then released in the powerstroke. From Houdusse, (2016).

Although research is ongoing and will likely result in further modifications of this account, it provides a model of how release of free energy is constrained to generate the activity of the myosin motor. The research reveals that muscle contraction or even the cycle of myosin engagements with actin are not primitive activities, but ones that can be explained in terms of the constraints on the free energy released in the hydrolysis of ATP.

3.2: Explaining the Activity of Kinesins

As with myosins, explaining the activity of kinesins required understanding how chemical energy from ATP is translated into mechanical energy in the kinesin molecule, resulting in this case, in the movement of one kinesin head in front of the other. After the discovery and initial characterization of kinesin, researchers assumed it was unlikely that it would operate in the same manner as myosin. First, kinesin is about half the size of myosin. Second, while a myosin generates a single power-stroke while attached to actin and then dissociates, an individual kinesin takes on the order of 100 steps along a microtubule before dissociating. Third, initial genetic sequencing of the two molecules did not suggest any homologies between the molecules. The research that ensued, however, resulted in a remarkably similar account of kinesin movement, one involving the constrained release of chemical energy from ATP, resulting in characteristic changes in kinesin's conformation which constitute its stepping activity.

The first step in working out the mechanism of kinesin movement was taken when Kull, Sablin, Lau, Fletterick, and Vale (1996) identified the crystal structure of kinesin bound to ADP and showed it to be similar to that which Rayment had identified for myosin just a couple years earlier. Using the three-dimensional structure as a guide, researchers then discovered homologous sequences in myosin and kinesin (Kull et al., 1998; Vale & Milligan, 2000), which

provided insight into the parts of the kinesin molecule involved in binding the γ-phosphate of the nucleotide.

The activity of kinesin in response to release of free energy is different from that of myosin, and this requires explanation. Research showed that it is due to differences in the way in which energy release is constrained. Kinesin does not have the elongated lever arm found in myosin. Instead, each head is connected to a neck linker which then connects via a common coiled-coil to cargo (Kozielski et al., 1997). Comparing crystal structures of kinesin bound to ADP in rat (Kozielski et al., 1997) and human (Kull et al., 1996) revealed a difference in the position of the neck linker. Rather than assuming that it was a species difference, researchers inferred a difference in the state of the motor molecule when it was crystalized. Moreover, they hypothesized that the neck linker played a similar role to the lever arm in myosin—its movement resulted from the way in which free energy released in ATP hydrolysis exerts forces that alter the conformation of the molecule.

Unable, at the time, to solve the crystal structure of kinesin in different states of ATP hydrolysis, Rice et al. (1999) instead used spectral analysis to show different conformations of the neck linker when kinesins are in different nucleotide and microtubule binding states. These researchers concluded that the neck linker is generally in an unstructured and so flexible state and becomes more ordered and immobile when the microtubule bound kinesin binds ATP (see step 5 in Figure 3.6 in which the position of the linker has changed to pointing forward from the head that is now in the rear). As a result of being in this structured state, the kinesin head exercises a force on the other head, moving it forward. With fluorescence resonance energy transfer (FRET), they measured the distance between specific residues and concluded that the

neck linker docks onto the catalytic core of the kinesin, a proposal they further supported with cryo-EM.

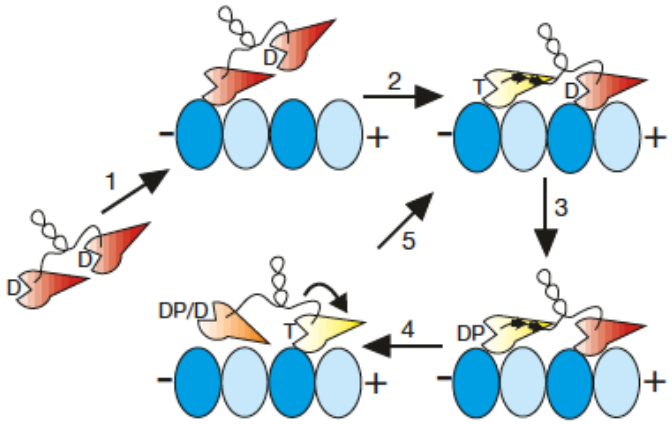

Figure 3.6: Changes in linker docking as a kinesin walks. From Rice et al. (1999).

In the last decade, with the development of high-resolution X-ray crystallography (resolution to 2.2 Å) and cryo-electron microscopy (resolution to 6 Å), researchers obtained images of both unbound kinesin and kinesin bound to tubulin and ATP or ADP that provided a clear image of how energy was transformed in kinesins. Comparing images of kinesin in different states, Cao et al. (2014) concluded that "the kinesin structural changes along the nucleotide cycle are well described by rigid-body movements of three motor subdomains": the switch 1/2 subdomain, the P-loop subdomain, and the tubulin binding subdomain. In particular, the crystal structures reveal that when ATP binds to the P-loop, as shown by the **A** with three connected back circles in the top portion of Figure 3.7B, the P-loop (orange triangle) and switches 1 and 2 (blue rectangle) align so that a place is opened for the neck-linker (red circle) to dock. This is due in large part to the fact that the first residue of the P-loop, an isoleucine, gets buried in the cavity. This creates the configuration that catalyzes ATP hydrolysis. The hydrolysis and expulsion of Pi (while still bound to ATP) exerts a force that causes the P-loop and two

switches to mechanically reconfigure, resulting in the closure of the docking site and the kinesin detaching from the microtubule. While detached, the docking of the other head thrusts this head forward. Once it expels ADP, this head can again bind to the microtubule, now at a location further along the microtubule. Although this changes the conformation of the P-loop and the switches, the neck-linker docking site remains blocked. It only opens again when a new molecule of ATP is bound (Wang, Cao, Wang, Gigant, & Knossow, 2015).

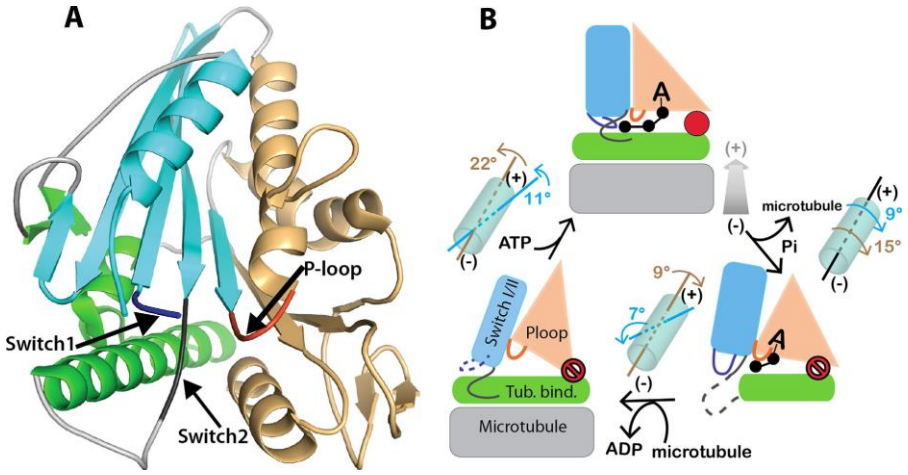

Figure 3.7: Ribbon diagram of kinesin showing the location of switches 1 and 2 and the p-loop (left) and a proposed mechanism by which the movements generated by ATP binding and hydrolysis open and close a locus for neck linker docking (right). From (Wang et al., 2015)

As in the case of myosin, research is ongoing and, while supporting the general picture of how energy from ATP hydrolysis is constrained to force mechanism movement, has challenged the details. According to Sindelar and Liu (2017), new research by Milic, Andreasson, Hancock, and Block (2014) and Mickolajczyk et al. (2015) is generating "A quiet revolution in the kinesin field [that] has recently contradicted the longstanding idea that the forward step by one-head-bound kinesin is triggered by ATP binding, establishing that the forward step instead occurs after hydrolysis of ATP." This led Hancock (2016), to advance a new consensus model according to which binding to ATP by the forward head enhances the release of the trailing head from the

microtubule while hydrolysis results in docking of the linker and the forward movement of the previously trailing head.

Although this recent research has altered the details, the basic categories in terms of which the activity of kinesin motors are explained remains the same: the constrained hydrolysis of ATP results in the free energy released exercising force that alters the conformation in both heads of the kinesin motor, resulting in the activity of walking along microtubules.

3.3: Explaining the Activity of Motor Movement

The research on both kinesin and myosin discussed in this section resulted in accounts that explain their activities. In both cases, researchers showed how the free energy released in ATP hydrolysis results in conformational changes to the binding pocket. These changes in turn apply forces to other parts of the motor, including the site of actin or microtubule binding. The changes at the binding site are responsible for the cycle of binding and unbinding from actin or the microtubules. The conformation changes generated by hydrolysis at the ATP binding site are also communicated to other parts of the molecule. In myosin they build up tension that forces a rotation of the level arm, which is then released in the powerstroke that pulls myosin along the attached actin. In the case of kinesin, force is applied to the neck linker that then serves to move the rear head in front where it finds a new binding site. Thus, both motors generate motion as a result of ATP hydrolysis in a constrained pocket creating forces that alter other parts of the motors. The differences in the activity of the two motors result from the different constraints imposed by the structural differences in the other parts of the molecules.

Section 4: Explaining Activities in Biological Mechanisms

On the standard account of mechanistic explanation, scientists explain a phenomenon by appealing to the entities and activities that constitute it. If they desire to explain the activities of one of the component entities, they repeat the process. Science that has followed this procedure has been enormously productive. Nothing in this paper is intended to downplay the contributions of such mechanistic research or philosophical accounts of it. (Below, though, we argue that an important addition to such accounts is to identify the source of free energy on which activities depend). Yet, at some point, such research typically bottoms out. As the new mechanists have discussed, this often occurs when researchers lack the tools or the interest in explaining the activities of the components they have identified. These are simply accepted. This process leaves the active nature of activities unexplained. Dualists such as MDC are happy to leave matters there. This is unsatisfying, however, if one wishes to understand how mechanisms are active.

The research we have discussed in the previous section identifies a way in which biologists have gone further and explained activities of mechanisms in more fundamental terms. The first step is to recognize that mechanisms are only active when free energy is employed in them. This requirement has not been emphasized in the accounts of the new mechanists, but it is fundamental. A mechanism without free energy will not perform work. Any movement will be due to external forces impinging on them or to thermal noise (Brownian motion). When there is a source of free energy in the mechanism, it can generate activity. Once one focuses on the need for free energy for a mechanism to perform activities, one can take the second step. What happens to the free energy depends on how it is constrained. If not constrained, it simply dissipates, and entropy increases, without work being performed. When it is constrained, work can be performed, with the nature of that work depending on the constraints imposed. As

illustrated in the case of molecular motors, constraints are provided by the physical parts of the mechanism and the way they are organized. As a result of these organized parts, free energy release is constrained in a particular manner, giving rise to the activities of the different parts.

As we have shown, mechanistic explanations of molecular motors appeal to energy and to the role parts of mechanisms play in constraining its release. We thus advocate for incorporating these into the mechanistic framework. The fundamental roles each performs is illustrated by a simple example from classical mechanics. At the core of Newtonian physics is the idea of force acting on objects. Consider the rectangular object shown in the 2D space on the left of figure 8. It has 3 degrees of freedom: it can move along the X and Y axes and can rotate. If a force is applied to it, it can move in any of these directions. The same object is shown on the right connected to a hinge. The hinge is a constraint on the movement of the rectangle: it reduces the object's degrees of freedom. Now it can only rotate when a force is applied to it. As articulated by Hooker (2013), constraints are both limiting (the rectangle can no longer move along the x axis) and enabling (it would have been difficult to apply a force that would get the rectangle to rotate counterclockwise and not along the x or y-axis in the situation on the left. When the rectangle is constrained by the hinge it is relatively easy: simply apply a force anywhere along the bottom edge of the rectangle.

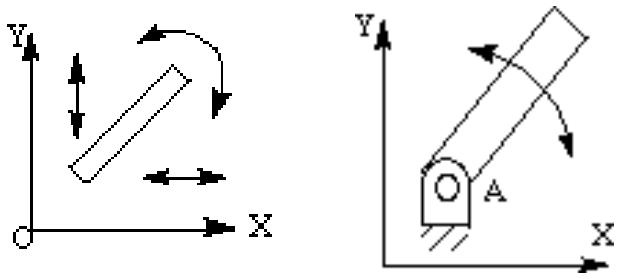

Figure 3.8: Illustration of A. unconstrained object subject to three degrees of freedom and B. a constrained object, limited to one degree of freedom. From https://www.cs.cmu.edu/~rapidproto/mechanisms/chpt4.html

The forces appealed to in Newtonian physics result from free energy. Imagine that the angle labeled A in figure 8 is a nucleotide binding pocket. If ATP hydrolysis occurs in that pocket, the chemical energy stored in the ATP molecule is translated into a mechanical force applied to the rectangle. This shows up as an activity—rotating. What we have here, then, is an entity engaging in an activity. But, further, we have an account of the means by which the rectangular object rotates—an account in terms of energetics and constraints. We draw this same lesson from the case of molecular motors research. Figure 3.8 presents in a very simplified form the basic explanatory principles appealed to in the explanations of the molecular motors and illustrated in (Figures 3.4, 3.5, and 3.7). The chemical energy released in the nucleotide binding pocket in each motor is translated into mechanical forces which alter the shape of the molecule. The particular constraints result in movements that constitute the activity of stepping. Given the greater number of parts acting as constraints, the kinematic activity in the molecular motors is in obvious ways much more complex than what is represented in Figure 3.8. Nonetheless, the basic explanatory principles are the same—energetics and constraints.

One might worry that in appealing to free energy to explain activities, we have replaced a dualism of entities and activities with one of constraints and free energy. In one sense, we have: both free energy and constraints are required in the explanation of an activity. But the need for free energy is well-recognized in physics. The principles of thermodynamics are central to understanding what happens in the universe. A dualism of matter and energy is forced on us by physics. As a "descriptive ontology of science" (Illari and Williamson 2015), such a dualism is well motivated. The category of activities, however, is largely a philosophical invention. When MDC introduce activities, they appeal to examples ("fitting, turning, opening, colliding, bending and pushing . . . ") to illustrate them. Moreover, these activities prompt questions: Why does this entity turn or open? What enables one entity to push another? Invoking activities as a distinct ontological category does not advance us towards an explanation of these activities—it simply reifies the fact that mechanistic components are active. The different ways in which free energy is constrained provide answers to these questions in terms of a category already required to explain everything that happens in the physical universe.

To be clear, however, we are not arguing for the elimination of activities as an analytic category in terms of which to explicate the structure of explanation in the biological sciences. Further, we are not arguing that mechanistic explanations that appeal to activities and do not explain them in terms of free energy are therefore explanatorily deficient. Entities in mechanisms do engage in activities and characterizing these activities allows one to explain how a mechanism in which these activities occur gives rise to a phenomenon. We are simply showing that activities are subject to further analysis and this analysis dispels the metaphysical mystery that surrounds activities when as they are characterized as ontologically fundamental.

On the traditional account, activities in a mechanism at one level are explained in terms of the entities and activities of a mechanism at a lower explanatory level. When researchers explain activities in terms of energetics and constraints, are they likewise doing so at a lower explanatory level? We think not. The entities into which the molecules are decomposed (e.g., nucleotide binding site, actin or microtubule binding site, neck linker, lever arm) and the activities ascribed to each of them (e.g., binding and releasing nucleotides, actin, or the microtubule, moving the lever arm or the neck linker) are not further decomposed into more basic entities performing more basic activities. Rather, for the purpose of explaining the activities of these entities, the entities themselves are construed as a set of constraints that respond in characteristic ways as free energy flows through them. The forces applied to the binding sites or to the linker, etc., just are the constrained release of free energy. What one has done is redescribed basic activities in terms of constrained release of free energy and thereby explained why they are active.

We have focused our case on molecular motors, and one might question whether the account of activities in molecular motors generalizes. We offer two reasons to think they are not an exceptional case. First, in addition to contraction of muscles and transport along microtubules, molecular motors perform many other activities in cells. Dyneins, for example, figure in both the movement of cilia and in positioning of chromosomes during cell division. The synthesis of ATP in the mitochondrion relies on the F_0F_1-ATP synthase molecular motor operating in reverse—using the free energy released from a proton gradient to turn a rotor that positions ADP and Pi appropriately to form ATP. The synthesis of microtubules depends on a molecular motor that uses GTP rather than ATP. Numerous nucleic acid motors, including RNA polymerase, DNA polymerase, and the ribosome, figure in gene expression. Far from being an unusual biological

mechanism, biological motors constitute a large category of biological mechanisms responsible for many activities of cells.

Second, a host of other cell mechanisms that are not generally classed as motors but that are critical to the functioning of cells rely on ATP or GTP as their source of free energy. For example, maintenance of appropriate concentrations of ion and proton gradients across membranes is critical for a variety of cell activities. These rely on pumps or transporters that utilize ATP or GTP to move ions or protons across the cell membrane against a concentration gradient. Perhaps the best known of these is the sodium-potassium pump that transports three sodium ions out of the cell and two potassium ions into the cell. In this case, binding and hydrolysis of ATP phosphorylates the pump protein, altering the conformation of the molecule so as to release sodium ions outside the cell and binding potassium ions. Binding potassium reverses the conformation change, resulting in release of potassium inside the cell and again binding sodium.

We have pitched our account of how to explain activities within mechanisms at the foundational level at which mechanistic explanations in biology bottom out. It is at this level where the chemical energy produced in metabolism gets translated into mechanical energy. The research on molecular motors we have discussed demonstrates directly how to explain activities in terms of energetics and constraints. However, energetics and constraints are also relevant to understanding mechanisms at higher levels of organization—ones traditionally explicated in terms of entities and activities. Even if not overtly explained in terms of energetics and constraints, ultimately, at any level, the activities of a mechanism are the result of free energy being constrained to perform work. Accounts of how this occurs in specific mechanisms will vary from mechanism to mechanism but, across all cases, energetics and constraints provide the

fundamental terms in which the mechanism's activities as such can be understood. In short, bringing energetics and constraints into the mechanistic fold vindicates, philosophically, the appeal to activities even in mechanistic explanations that do not explicitly cash out their appeals to activities in energetic terms. To use an analogy, just as, some argue, Darwin legitimized talk of design in biology by showing how it can be brought about by means that pass naturalistic muster, we claim to have legitimized talk of mechanistic activities by showing how they can be analyzed in terms of energetics and constraints.

What does it look like to explain the activities of mechanistic entities at levels of organization higher than the bottom-out level in energetic terms? This involves identifying the point at which free energy enters the system at that level. We illustrate this by returning to the case of muscle movement resulting from ATP hydrolysis by myosin. The work performed by the contraction of muscle cells depends on the specific tendons that attach muscles to bones and ligaments that attach bones to one another. These further constrain the energy released in the action of the motor. The ability of myosin to bind to actin depends upon release of Ca^{++} from the sarcoplasmic reticulum in response to action potentials generated in muscles as a result of the action potentials in nerves reaching the neural muscular junction. These in turn depend upon activity in the central nervous system that, in part, depends on events in the organism's environment. Both of these require further sources of free energy made available by pumps that create the Ca^{++} gradient across the sarcoplasmic membrane and pumps that create ion gradients over the membranes of neurons. Emphasizing the role of the constrained release of free energy in explaining activities applies across all levels in the mechanistic hierarchy. Higher-level activities, just as those at the bottom-out level, depend upon the release of energy. Higher-level entities also

constrain those at the bottom level, determining how energy released in molecular motors, ion pumps, etc. results in activities at higher levels.

As we mentioned, explanations of the activities of higher levels can, especially in light of our philosophical vindication of the category of activities, reference activities at the next lower level without themselves revealing *how* free energy gives rise to those activities. Accordingly, mechanistic explanation above the foundational level where the constrained release of free energy results in movement can be conducted in the standard manner characterized by the new mechanists. Yet, we contend that even these accounts would benefit from attending to *where* free energy is supplied to these mechanisms. On the one hand, it will help localize where the work is performed that explains the activity of the mechanism. On the other, it will provide a reference point for understanding the operation of the mechanism. Machamer et al. (2000) present mechanisms as operating from "start or set-up to finish or termination conditions" (p. 3) Given the cyclic organization found in many biological mechanisms, one can question whether there are principled start or termination conditions. The entry of free energy provides a principled starting point for analyzing the activity in the mechanism and the products of the work performed by the constrained release of energy provides a plausible set of termination conditions. Moreover, even if one does not directly track energy transduction in developing one's account of the mechanism, one can plausibly infer that these activities result from the constrained flow of free energy through the mechanism and so organize the account of the mechanism around that flow.

Conclusion

The components of mechanisms are active. The standard account of mechanistic explanation explains activities in terms of activities and does not offer an explanation other than

to appeal to other activities. To illustrate how scientists in fact provide explanations of activity without appeal to other activities, we have focused on two molecular motors, myosins and kinesins. The research on both motors that we analyzed in section 2 proceeded in the manner characterized by the new mechanists, ultimately showing how stages in ATP hydrolysis corresponded to steps in the stepping behavior of both motors. But, as we showed in section 3, research on molecular motors has taken an additional step, showing where free energy is released from hydrolysis of ATP and how it leads to conformation changes in other parts of the molecule that constitute the stepping. The motor provides a set of constraints in which free energy is directed into forces that generate movements that corresponds to the activities of the motor. In section 4 we flesh out the framework advanced in our analysis—appealing to free energy and its constrained release to explain activities. As a result of the constrained release of free energy, biological mechanisms are active, but their activities are not primitive posits. They can be explained.

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Chapter 4: Discovering Autoinhibition as a Design Principle for the Control of Biological Mechanisms

Section 1: Introduction

Much scientific research on biological mechanisms focuses on how they account for phenomena—e.g., the division of a cell, the contraction of a muscle, the synthesis or degradation of a protein. From this perspective, it is surprising that many molecular mechanisms in biology are organized so that they autoinhibit—that the parts of the mechanism act on others in a manner that renders the mechanism unable to perform the phenomenon for which it is responsible. Autoinhibition involves *intra*molecular interactions between distinct domains of a molecule such that one region impedes the activity of another. For a mechanism to generate the phenomenon with which it is identified, it must be released from autoinhibition through *inter*molecular interactions between the target protein and binding partners that serve to alter the protein's conformation, activating the formerly inhibited domains (Pufall & Graves, 2002).

We argue that mechanisms operating to inhibit their ability to produce the phenomenon with which they are identified should be viewed as instantiating a design principle: a commonly implemented pattern of organization that can be described generally and realized in different molecular implementations. In section 2 we introduce recent discussions of design principles, including discussions of how they provide generalized principles that can be invoked in explanations, including mechanistic explanations. While there has been discussion of the explanatory roles of design principles in both philosophy and various areas of biology, there has been relatively little discussion of how scientists discover design principles. We focus on the discovery of autoinhibition, using the discovery of the autoinhibition of the molecular motors kinesin and cytoplasmic dynein as examples.

Although, as Pufall and Graves demonstrate, autoinhibition is a design principle widely instantiated in biological mechanisms, it is typically not discovered in the same manner as new mechanists have described the discovery of mechanisms—starting with the phenomenon, identifying the responsible mechanism, and then figuring out its components and how they are organized (Illari & Williamson, 2012). As the cases of kinesin and dynein show, that these mechanisms inhibit themselves was only recognized after they were identified as the mechanisms responsible for cellular phenomena such as axonal transport. Moreover, autoinhibition was concealed by the very experimental procedures widely used to study molecular mechanisms. Such procedures are designed to reliably produce the phenomenon in which researchers are interested, which requires overriding the processes resulting in autoinhibition. Only as broader inquiry was proceeding—inquiry using other experimental tools— did researchers studying both kinesin and dynein come to recognize that much of the time these mechanisms generated a different phenomenon, autoinhibition.

As background to research leading to the discovery that both kinesin and dynein inhibit themselves, in section 3, we discuss the research leading to the discovery of kinesin and cytoplasmic dynein as the motors responsible for axonal transport and explanations of their ability to produce movement from the hydrolysis of ATP. This research proceeded in the manner the new mechanists have characterized: researchers identified phenomena of interest and associated it with a mechanism, then decomposed the mechanism into relevant parts and determined how they operate in an organized fashion so as to generate movement. In sections 4 and 5, we turn to how researchers discovered that the motors autoinhibit when not needed for transport. The paths to discovering that kinesin and dynein autoinhibit were quite different. In the case of kinesin, it occurred shortly after the discovery of the protein itself. In the case of dynein,

it took considerably longer. We take advantage of the differences in the two cases to further elaborate on the reasoning that goes into piecing together accounts of how the proteins act to inhibit themselves and how other processes in the cell release them from that state and enable them to perform their activities of transporting cargo.

In section 6 we consider the implications of the case studies of kinesin and dynein for the understanding of mechanisms. As we have noted, the discovery that these mechanisms autoinhibit followed on their discovery as the mechanisms that generated the motility needed for axonal transport. This not only reverses the typical pattern of mechanism discovery but also provides a different perspective on the relation between mechanisms and phenomena, challenging the one phenomenon-one mechanism principle that most mechanists have adopted. In mechanisms that autoinhibit, the same mechanism is involved in different phenomena. Which phenomenon they engage in depends on how they are controlled. Since autoinhibition is, as we argue, a design principle widely implemented in biological mechanisms, this critical point against standard accounts of mechanism derives not only from our case study (which we use to illustrate the distinctive pattern involved in the discovery of autoinhibition) but from the whole suite of autoinhibitory mechanisms in the cell. The ubiquity of autoinhibition, in turn, motivates an alternative philosophical understanding of mechanisms that countenances how mechanisms can behave differently under different conditions of control. To understand how control processes act on mechanisms and determine what phenomenon they produce, we draw on a reconceptualization of mechanisms as systems that constrain flows of free energy. We conclude in section 7.

Section 2: Autoinhibition as a Design Principle

A common theme in philosophy of biology is that the biological world is contingent and accordingly that there are no laws in biology. Smart (1963) argues that biological phenomena lacked the regularity required to be subsumed under laws. Beatty (1995) argues that biological systems, as the products of evolution, are contingent; as a result, any generalizations that are found do not qualify as laws. The lack of recognizable laws was a factor leading Bechtel and Richardson (1993/2010) to reject the D-N model of explanation and argue that many explanations in biology took the form of identifying mechanisms.[35] Resisting this tradition of denying laws in biology, Green (2015) draws upon examples in systems biology to show that what systems biologists refer to as *design principles* provide generalizations that can be invoked in biological explanations. She quotes Ma, Trusina, El-Samad, and Lim's (2009) characterization of design principles as "organizational rules that underlie what networks can achieve particular biological functions" (637).

To highlight a system's organization, a common strategy in systems biology is to represent the entities and interactions of components of a system as nodes and edges in a network. Such a representation is indifferent to the identities of particular components as these are ancillary to the pattern of organization represented in the network. In his pioneering research using network representations, Alon and his collaborators (Milo et al., 2002; Shen-Orr, Milo, Mangan, & Alon, 2002) identified numerous particular subnetworks within larger networks specifiable in this way. Each subnetwork involves two to four nodes connected in the same manner, which Alon and colleagues referred to as *motifs*. For instance, Figure 1A illustrates a *coherent feedforward network*. It consists of three nodes, labeled X, Y, and Z (S in the input to the motif), in which node X activates node Z both directly and by activating Y which in turns

[35] Mechanisms are intended to generalize over many instances in which a phenomenon is produced, but there may be no generalizations across mechanisms responsible for different phenomena.

activates Z. Using Boolean modeling, they showed that if node Z acted as an and-gate and if it took time for each node to respond to inputs from the previous node, such a motif would act as a persistence detector—node Z would only become active if input S was maintained sufficiently long for Y to become active and for both X and Y to send outputs to Z.[36] An important feature of motifs is that they abstract over details about the identity of X, Y, and Z; as a result, the analysis of how the motif functions explains what happens in all instantiations. Figure 1B shows an even simpler motif explored by Tyson and Novák (2010)—a double negative feedback loop which, with appropriate parameters, enables switching between two stable regimes. In this case, the need for appropriate parameters limits the applicability of the motif, but it still generalizes over a wide domain.

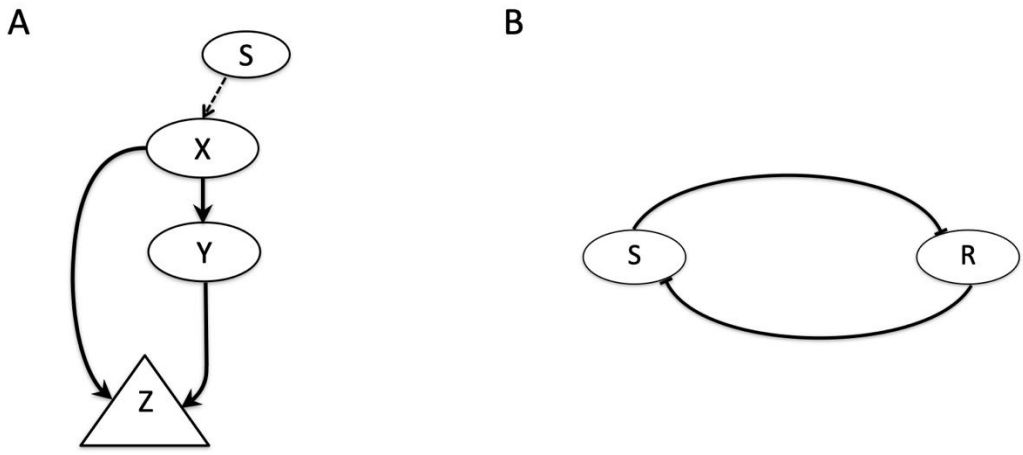

Figure 4.1: A. A coherent feedforward loop motif. B. A double negative feedback loop motif.

[36] As Alon's use of Boolean modeling makes clear, motifs and other design principles are often analyzed in computational terms. As in the case of the double-negative feedback loop discussed below, such modeling reveals the specific conditions (reflected in parameters in the computational model) under which the design principle will realize the specific effect. In many cases, qualitative analysis, such as provided in the text, suffices to appreciate the effect. We treat design principles as patterns of organization that can be analyzed either qualitatively or quantitatively.

Motifs illustrate fundamental features of design principles—they are ways of organizing components in which the resulting function does not depend on the specific features of the entities realizing the nodes; as a result, the motif itself can be appealed to in explanations of a diverse set of phenomena. Green, Levy, and Bechtel (2015, p. 16) capture this in their characterization of design principles as "patterns of organization that can be specified abstractly, supplying an explanation for a given behavior that occurs across a range of cases in which the organizational pattern is realized."

The word *design* is closely associated with the idea of a designer. Green et al. emphasize, however, that design principles can arise through the course of evolution without a designer. They need not even be adaptations—the product of natural selection. Nonetheless, they may be promoted by natural selection—one can view natural selection as exploring different designs. In this spirit, Lim, Lee, and Tang (2013, p. 202) characterize design principles as "archetypal classes"—"common patterns for how diverse and complex regulatory [systems] . . . achieve a particular function." When considering evolution, they can serve as "attractors" in the "underlying landscape within which evolution can explore." As attractors, Lim et al. characterize them as patterns that would regularly appear "if one could hypothetically replay evolution over repeatedly."

Systems biology is not the only area of biology invoking design principles. They are also employed in cell and molecular biology. One of the examples of design principles that Lim et al. present is the common organization that Steitz (1999) showed to be exhibited by different DNA polymerases (Figure 2). In this case, it is the common features of the organization of the different proteins that is viewed as a design principle and is invoked to explain the functioning of the protein as a polymerase. Cell biologists Rafelski and Marshall (2008) similarly appeal to abstract

features of mechanisms to propose ways in which "mechanisms pattern the architecture of the cell." They explain that they borrow the term *design principle* from engineering to designate "simple rules that, when followed in the design of a machine, ensure or at least increase the likelihood of proper assembly or function" (593). In discussing a design that could control the size of developing cellular structures, for instance, what they term *molecular rulers* have lengths corresponding to the desired length of the structures of which it controls the development. For example, the gene *H* product dictates the length of the λ-phage tail by attaching to the growing tail and preventing the action of a growth-terminating factor until the tail outgrows the "ruler" (gene *H* product).

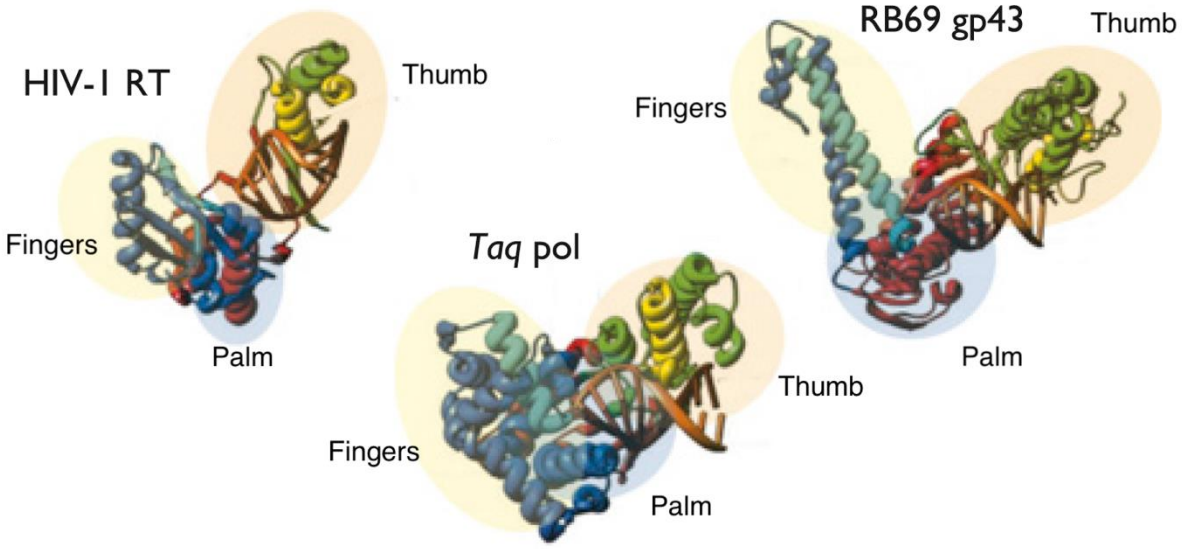

Figure 4.2: A structural design principle illustrated in three DNA polymerases. Adapted from Steitz (1999); reproduced under Creative Commons CC BY license.

Yet another field in which design principles are invoked is synthetic biology. Stein and Alexandrov (2015), for example, invoke actual protein switches found in cells as a basis for engineering switches to perform new functions. One of the design principles they develop is of particular relevance for our discussion below—autoinhibition. They present a cartoon (Figure 3)

to illustrate the design principle through which a ligand (L) can activate a switch, releasing the autoinhibitory domain AI, rendering protein A active. Like network diagrams, by using abstract shapes a cartoon like this makes clear that the design can be instantiated by different components.

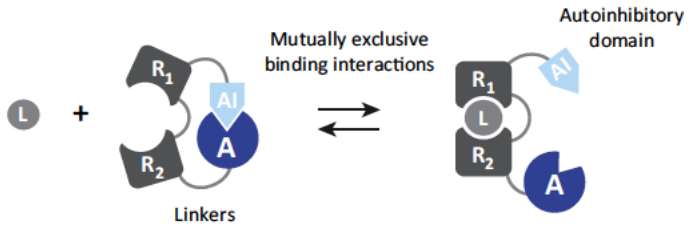

Figure 4.3: A ligand binding to a switch can release a target from autoinhibition. Adapted from Stein and Alexandrov (2015) with permission from Elsevier.

As emphasized by Green (2015), one reason design principles are philosophically important is that they make "room for generality in biology." This virtue is well captured in Salvador's (2008) discussion of the implication of Alon's identification of motifs:

> molecular biology might one day be structured around a number of simple laws or principles whose understanding hinges largely on engineering considerations similar to those applying to human designed circuits. The major breakthroughs in the exact sciences occurred when the main regularities (laws) were discovered and then explained. From this process ensued the predictive power that earned these sciences the qualifier "exact," which still sets them apart from biology. If a similar process is nowadays taking place in molecular biology this is largely through the discovery and explanation of design principles (193).

Our brief discussion illustrates a wide range of designs that biologists in different domains of biology refer to as design principles. We turn now to the specific example on which we focus, autoinhibition. Treating it as a widespread phenomenon, Pufall and Graves (2002) characterize autoinhibition abstractly: "intramolecular interactions between separable elements within a single polypeptide provide a common regulatory strategy [in which] one region of a

protein interacts with another to negatively regulate its activity" (422). The intramolecular interactions are illustrated in a cartoon fashion in Figure 4 in which a domain of a protein inhibiting its activity is indicated by an edge-ended line between an oval representing the inhibitory domain and another representing the activity of the protein. Since there are conditions in which the activity of the protein is required, the figure also identifies three ways in which an inhibited protein can be released from autoinhibition through intermolecular activities.

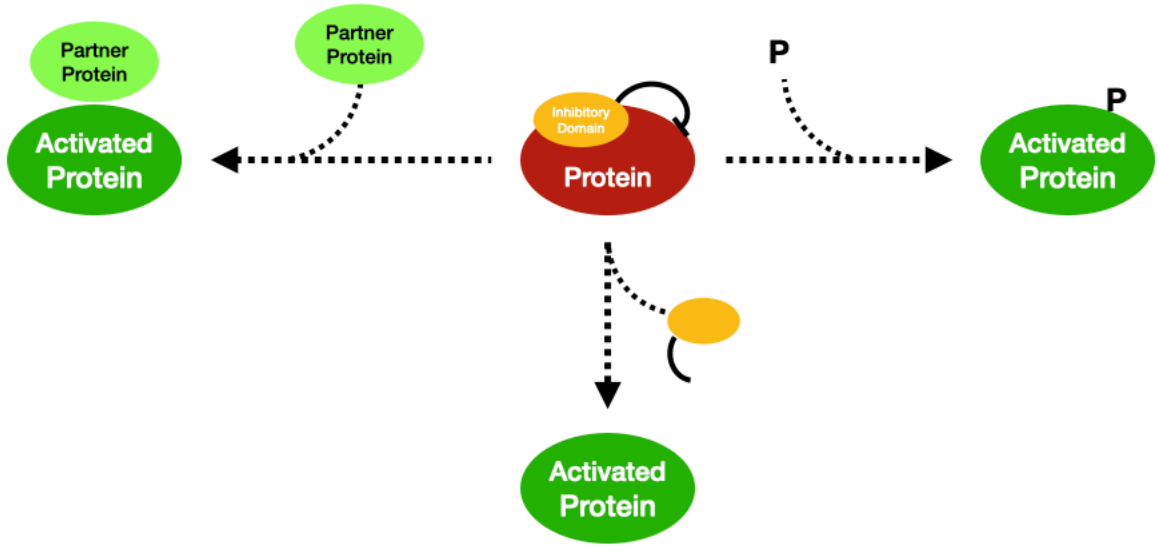

Figure 4.4: The inhibited state of a protein (center of diagram) involves an intramolecular interaction which prevents it from performing its activity. Activation of a protein requires intermolecular interactions between a signal and the autoinhibiting protein. Three ways in which this can happen are illustrated. Notice that the abstract specification of the principle makes no reference to any particular proteins.

Without labeling it a design principle, Pufall and Graves argue that autoinhibition is a "common regulatory strategy to modulate protein function." In support of this claim, they provide a detailed account of seven examples and list over thirty other instances. This frequent occurrence

suggests that it is what Lim et al. characterized as an evolutionary attractor and appropriately characterized as a design principle.

Autoinhibition is useful in explaining the contribution of proteins to cell activities. The activities performed by proteins are invoked in explaining how cells generate different phenomena. But most phenomena (e.g., cell division, synthesis of proteins, autophagy), are only useful to the cell on some occasions and at other times are detrimental. For instance, cell division is useful to construct a multicellular organism, but unconstrained cell division is a feature of cancer. Employing a design in which an intramolecular interaction inhibits the ability of the protein to perform its activity ensures that it will not act except when a signal specifically releases it from autoinhibition.

As we discussed, both philosophers and biologists have articulated the significance of design principles for understanding explanation in biology. In the spirit of mechanists concerned with discovery (Bechtel & Richardson, 1993/2010; Craver & Darden, 2013), we take up the question of how they are discovered. The example of autoinhibition is useful for this purpose since experiments are designed to enable mechanisms to operate and hence involve procedures that effectively release proteins from autoinhibition whether researchers understand this explicitly or not. As Pufall and Graves note, the assays used to study protein function frequently "bias" researchers to focus on the active state of the protein and not notice that regions of it may serve an autoinhibitory function.[37] This poses the question: how do researchers come to notice instances of autoinhibition? To address this question, we turn to research on two molecular mechanisms—the motor proteins kinesin and dynein. The paths to discovering that kinesin and dynein autoinhibit were quite different. The discovery occurred relatively quickly in the case of

[37] Pufall and Graves offer this as a reason to "predict that there are undoubtedly many more examples of autoinhibition to be discovered" (453).

kinesin—shortly after the discovery of the protein itself—while, in the case of dynein, discovering that it had this design took considerably longer. This difference across cases works in our favor, philosophically, as it helps more fully characterize the process and significance of the discovery of autoinhibition. In both cases, the discovery that the mechanism autoinhibits followed on the discovery of the mechanism itself. Accordingly, we turn to the discovery of kinesin and dynein in the next section, reserving to sections 4 and 5 the analysis of how each was found to autoinhibit.

Section 3: Discovering the motors responsible for axonal transport

In this section we describe how researchers, starting from observations of fast axonal transport, discovered the responsible mechanisms. This involved identifying two mechanisms they took to be loci of control (Bechtel & Richardson, 1993/2010) for that phenomenon—the molecular motors kinesin and dynein, and characterizing how, in each case, their movement patterns drive axonal transport. The movement pattern of the motors now became the phenomenon to be explained. To do so, researchers decomposed them into their organized parts and operations and demonstrated that the proposed mechanism can generate the phenomenon of motility. This pattern of discovery is familiar to mechanist philosophy of science according to which researchers identify a phenomenon of interest and, decomposing it into parts and localizing functions to those parts, specify the mechanism responsible for it (Illari & Williamson, 2012).

Fast axonal transport was first identified through research on nerve regeneration that began during World War II. When researchers found that constricted axons swelled to two or three times their normal diameter at the point of constriction, they attributed the swelling to

material moving through the axoplasm and accumulating at the point of constriction. Using radioactive isotopes that became available after the war, investigators followed the movement of labeled material through the axon. By cutting out segments of the axons at different times, they were able to determine when various molecules reached each section. Such experiments revealed that transport occurs in two directions, toward and away from the center of the cell. These were dubbed *anterograde* and *retrograde* axonal transport respectively (Grafstein & Forman, 1980). Attaching video cameras to microscopes, Allen, Allen, and Travis (1981) directly visualized the movement of radioactively tagged organelles and proteins and distinguished different rates at which cargo was transported. Even the slow transport they observed was faster than could be explained by diffusion. Accordingly, researchers began searching for the responsible mechanism. By extracting axoplasm out of axons and observing that particle transport still occurred, Brady, Lasek, and Allen (1982) concluded the mechanism resided in the cytoplasm and did not involve the plasma membrane.

Research during the same period had identified a cytoarchitecture within cells that consisted of microtubules, microfilaments and intermediate filaments. Microtubules are long (sometimes as long as 50 micrometers), hollow cylinders (approximately 25 nm in diameter), typically consisting of 13 protofilaments. Each protofilament is made of heterodimers of α- and β-tubulin proteins (Figure 5). Typically, microtubules are arranged in the cell a bit like the spokes of a wheel, extending from what is designated the "minus-ends" near the nucleus or centrosome of the cell to the "plus-ends" at the cell periphery. Schnapp, Vale, Scheetz and Reese (1985) showed that axonal transport occurred along microtubules by correlating images of vesicles moving along filaments under a video microscope with electron micrographs of the same material. Under EM, these researchers were able to identify the filaments along which vesicle

movement occurred as single microtubules. While electron microscopy enabled these researchers to identify microtubules as the tracks, it did not identify the motor driving movement along those tracks.

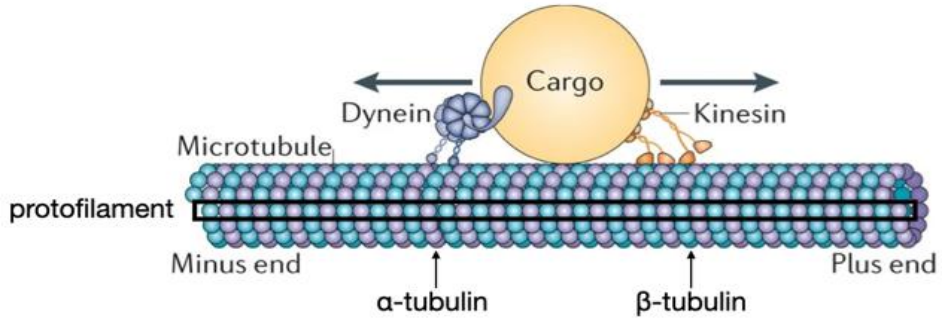

Figure 4.5: Microtubule with dynein moving cargo toward the minus end and kinesin toward the plus end. Adapted from Hancock (2014) with permission from Springer Nature.

Recognizing that movement faster than that achieved through diffusion required a source of energy and that this would most likely be provided by hydrolysis of ATP, Vale and his colleagues initiated a search among proteins associated with microtubules for those that hydrolyze ATP (ATPases). Initially expecting the ATPase to be attached to transported vesicles, they used centrifugation to purify microtubules and vesicles from axons and, combining them with the soluble fraction from the centrifugation process on glass coverslips, observed the vesicles to move, like transported cargo, along the glass coverslips. Running a control experiment to ensure that they were observing vesicles and not aggregated proteins, these researchers combined only the soluble fraction and microtubules on a glass coverslip. Since they believed the motor would be bound to vesicles, they anticipated no movement. To their surprise,

the microtubules began to slide over the coverslip. Now knowing that the motor was in the soluble fraction, they were able to isolate and characterize it, naming it *kinesin* (from the Greek word *kinein*, to move).[38] Kinesins turned out to constitute a large superfamily of proteins, more than 40 of which occur in mammals. Most kinesins transport cargo to the plus end of microtubules. We focus primarily on kinesin-1, the founding member of the superfamily, referring to it simply as *kinesin*.

In a preparation in which researchers had immobilized microtubules on glass coverslips and observed kinesin-bound beads moving along them, Vale et al. (1985) inhibited the activity of the kinesin and observed that minus-end directed transport still occurred along microtubules. They concluded that kinesin only drives anterograde movement and that another motor is responsible for retrograde movement. Vallee, Wall, Paschal, and Shpetner (1988) identified the second ATPase and, employing electron microscopy, demonstrated that it was "structurally equivalent" to axonemal dynein, a motor that had been identified 20 years earlier as responsible for movement of cilia (Gibbons & Rowe, 1965). The new dynein came to be known as *cytoplasmic dynein*.

With the identification of these two motor proteins, research proceeded on two fronts. First, researchers shifted their attention away from fast axonal transport and toward the detailed movement of the motors themselves, characterizing the stepping patterns of kinesin and dynein as they moved along the microtubules. Second, they developed mechanistic explanations for these stepping patterns, seeking to understand the means by which the motors step in the

[38] In successfully isolating kinesin, these researchers developed a novel technique that built on an earlier discovery that AMP-PNP, a non-hydrolyzable ATP analog, stopped transport along microtubules (Lasek & Brady, 1985). This led Vale and his team to use AMP-PNP to bind the as-yet-unidentified motors to microtubules and then purify the microtubules along with presumably, the attached motors. When the purified microtubules were treated with ATP to counter the effects of AMP-PNP, the material released was examined and found to contain a novel protein that, when combined with ATP and microtubules in a motility assay, caused microtubules to slide.

characteristic ways they do. The development of an innovative tool—the single-molecule motility assay—was crucial in studying kinesin movement in greater detail. Using purified kinesin and microtubules, Howard, Hudspeth and Vale (1989) reconstituted kinesin-driven motion by immobilizing single kinesin molecules "heads-up" on glass coverslips. This enabled them to observe, under a video microscope, single kinesin molecules pushing microtubules around. An alternative version flipped this geometry, immobilizing microtubules on glass coverslips and coating tiny plastic beads with kinesin. The movement of the beads was then visible as they were carried along the microtubule track by the kinesin motors. By analyzing the motion of these beads or microtubules, researchers were able to draw inferences to the stepping activities of the kinesin motor driving it. They determined, for instance, that it walked "processively," taking steps in which one of two "heads" remained attached to the microtubule while the other head moved, that it could take many steps before totally detaching, and that the two heads are asymmetric in their movement (Bollhagen 2021). A similar scenario played out in the case of dynein. Single molecule studies of dynein revealed that they also move processively, but that their walking was more erratic than that of kinesin, with occasional backwards and sidewise steps (Reck-Peterson et al., 2006; Qiu et al., 2012).

Even as research aimed at characterizing the motor's stepping patterns proceeded, investigations directed at explaining these activities was initiated. A first step was to determine the parts of the motor molecules. Kinesin was found to consist of two N-terminal heavy chains and two C-terminal light chains which bind to cargo (Bloom, Wagner, Pfister, & Brady, 1988; Scholey, Heuser, Yang, & Goldstein, 1989). From electron micrographs, Hirokawa et al. (1989) revealed that the heavy chains form an elongated coiled-coil which dimerizes at a "neck linker" into globular heads at one end and binds the two light chain tails at the other (Figure 6).

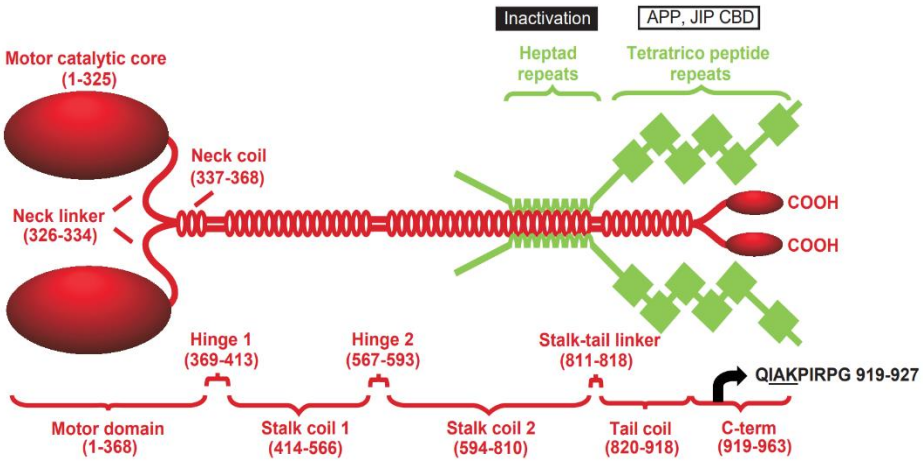

Figure 4.6: Structure of kinesin 1. Adapted from Schnapp (2003) with permission from Journal of Cell Science.

Higher resolution EM studies revealed that the globular heads contained the loci of ATP binding and hydrolysis and of microtubule binding. These heads became the foci in attempts to explain how the energy released in ATP hydrolysis generated motion. By crystalizing kinesin in different states of ATP hydrolysis, researchers demonstrated that the heads adopted different conformations before and after hydrolyzing ATP. Rice et al. (1999) developed a scenario according to which the conformation of the overall molecule changes as it binds ATP, hydrolyzes it, and then expels the resulting ADP and Pi. Among the consequences of these changes is that kinesin binds to and subsequently detaches from the microtubule. The conformation change also affects the linker that connects the two heads so that when one head is detached from the

microtubule, it is forced forward to where it binds to the next binding site towards the plus-end of the microtubule (Figure 7).[39]

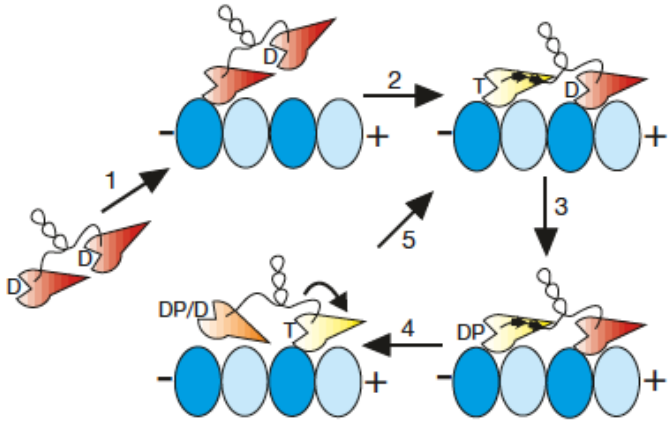

Figure 4.7: Mechanistic account of kinesin walking. Depending on whether the kinesin bound ATP (T), hydrolyzed it to ADP (DP if phosphate is still at the site, D once it has been jettisoned), each head is bound or released from the microtubule. The force exerted on the linker moves the trailing head, once free, ahead of the previously forward head, where it binds the microtubule again. Reproduced with permission from Springer Nature. Rice et al. (1999).

Similar findings revealed how dynein produces movement. Like kinesin, dynein is a dimer of two proteins, each of which contains a heavy chain that forms a globular head. However, studies of its structure revealed important differences (Neuwald, Aravind, Spouge, & Koonin, 1999). The motor domain in the globular head consists of a ring of six AAA+ (ATPases associated with cellular activities) modules, four of which are capable of hydrolyzing ATP (only the first produces the force used to move the motor). The microtubule-binding site is separated from the motor domain at the end of a coiled-coil stalk. Researchers have developed detailed models of how the conformation changes induced by ATP hydrolysis alter the configuration of

[39] For a detailed account of this research as well as research on a similar motor, myosin, and a discussion of how the mechanisms arrived at differ from standard new mechanist accounts of mechanisms, see Bechtel and Bollhagen (2021).

the head, which in turn alters the stalk so as to change whether the microtubule binding site can bind the microtubule (Figure 8). These models further describe how force generated by ATP hydrolysis is communicated to the linker that joins the two heads and propels movement towards the minus-end of the microtubule.

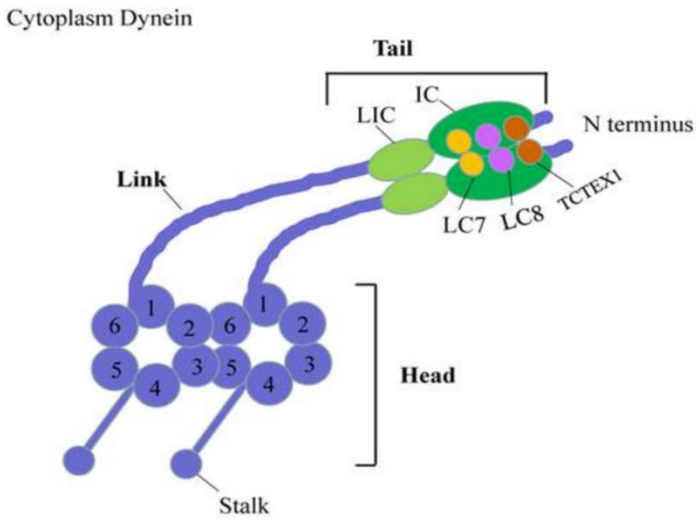

Figure 4.8: The structure of dynein. Reproduced under Creative Commons CC BY license from Xiao, Hu, Wei, and Tam (2016).

The research described in this section fits the accounts of discovery by the new mechanists according to which, a mechanism is sought to explain how a phenomenon is produced (Machamer, Darden, & Craver, 2000) or how some task is carried out (Bechtel & Richardson, 1993/2010). As Darden (2008) states, "identifying a puzzling phenomenon is the first step in an investigation of a mechanism." The phenomenon or task to be explained provides a "perspective" from which researchers can study the mechanisms underpinning them, deploying the heuristics and strategies mechanist philosophers have identified and characterized—e.g. decomposition and localization (Bechtel & Richardson, 1993/2010), schema instantiation and forward and backward chaining (Darden, 2008; Craver, 2007). The mechanism, once discovered,

is understood as the mechanism *for* the phenomenon or task the identification of which initiated the inquiry. In our case, the phenomenon of interest was, initially, axonal transport. Upon discovery of kinesin and dynein, their stepping patterns became the phenomena to be explained. These phenomena provided a perspective which was embodied in the single-molecule motility assay that enabled researchers to investigate the stepping patterns of the individual motor proteins. Researchers then decomposed the motors and pieced together accounts of the mechanisms *for* motor movement.

There is no question that the single-molecule motility assay was extremely productive in advancing mechanistic explanations of how kinesins and dyneins generate anterograde and retrograde movement respectively. The assay, however, is designed to make the motors generate movement, concealing the fact that much of the time the motors are unable to move as a result of inhibiting themselves. Recognizing that they instantiated the design principle of autoinhibition involved a shift away from the perspective in which movement is *the* phenomenon for which the motors are responsible to a perspective in which researchers could recognize that these motors autoinhibit and only produce motility when released from autoinhibition. From this new perspective, these motors are controlled by processes in the cell. Researchers arrived at this different perspective by different trajectories in the cases of kinesin and dynein; accordingly, we discuss them separately in the next two sections.

Section 4: Discovering Autoinhibition in Kinesin

In the previous section we described how Howard et al.'s single-molecule motility assay enabled researchers to establish that kinesins walk processively. We did not note that the researchers first attempt to show that a single kinesin was capable of moving a microtubule

failed to generate motion. The researchers offered two explanations for this failure: 1) either single kinesin molecules cannot move microtubules or 2) kinesin "denatures"—breaks, essentially—when it binds to the glass. Assuming the latter, these researchers pre-treated their coverslips with other proteins (tubulin and cytochrome c) to prevent the hypothesized denaturation. With the pre-treatment, they observed what they inferred to be microtubules sliding across single kinesins.

Another study published the same year advanced a different understanding of the pretreatment. Hisanaga et al. (1989) showed that most kinesins in cells are unattached to microtubules and exist in a "folded" conformation with their cargo-binding "tails" in close proximity to their MT-binding hydrolytic heads. The researchers found that when suspended in a buffer with high salt concentration, kinesins unfolded, assuming an extended conformation. Hackney, Levitt, and Suhan (1992) confirmed these findings and used them to account for a prior biochemical finding that purified kinesin motor domains with their tails removed hydrolyzed ATP faster than full length kinesin. Initially this was puzzling since it was not clear why the presence of the tail region would reduce the activity of the hydrolytic heads. Hackney et al. offered an explanation: the folded conformation, available only to the full-length kinesin, represents an autoinhibited state: by bringing the tail and head regions together both the ATPase and MT-binding sites become inaccessible (Figure 9). They concluded that the "folded conformation is enzymatically inhibited and may represent a soluble pool of the enzyme" (p. 8700). This explained why Howard et al. had to pretreat their coverslips. Rather than preventing denaturation, as Howard et al. had put it, the pre-treatment released kinesin from inhibiting itself, enabling MT-binding and uninhibited ATP hydrolysis. Hackney et al. hypothesized that a similar inhibition-releasing mechanism could operate *in vivo*.

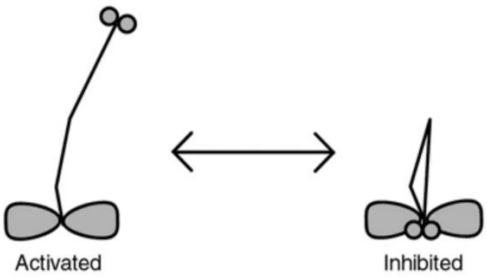

Figure 4.9: Basic "tail-inhibition" model. Reproduced with permission from Springer Nature. Coy, Hancock, Wagenbach, and Howard (1999).

The shift from the language of "denaturing" to that of "inhibiting"[40] marks an important shift in perspective. From the perspective embodied in the single-molecule motility assay, the target phenomenon is the movement of the motor. In order to study motor movement, techniques must generate movement reliably. From this perspective, a motor that is not generating movement is simply not producing its phenomenon. In short, it is "broken" or, "denatured." To think of a motor as inhibiting itself is to adopt a new perspective from which a motor that is not generating motion is, nonetheless, seen as functioning properly. The phenomenon it is generating is merely different from motility. Once a perspective on the motors is adopted which attributes to them a distinctive function—autoinhibition—inquiry can move in new directions.

First, once researchers adopted the perspective that, in addition to motility, kinesins engage in autoinhibition, they can make that a focus of inquiry. Coy et al. (1999), for instance, theorized about its physiological significance: if kinesins did not inhibit themselves, they would take futile, non-cargo carrying trips down microtubules, over-accumulate on microtubule tracks causing traffic jams, and wastefully hydrolyze ATP (back-of-the-envelope calculations suggested they

[40] Hackney et al. described part of kinesin inhibiting other parts, but did not use the term *autoinhibition*. Later publications that did use the term (Kaan, Hackney, & Kozielski, 2011; Verhey & Hammond, 2009) cite Hackney et al. as establishing that kinesins autoinhibited.

would do so at a rate comparable to the total metabolic rate of humans).[41] Other investigations filled in details of kinesin's autoinhibited conformation. A productive line of research drew upon Verhey et al.'s (1998) determination that the heptad repeats shown in Figure 6 are responsible for the binding of the kinesin heavy chains (KHCs) to the kinesin light chains (KLCs). The researchers further determined that the heptad repeats are necessary, but not sufficient, for inhibition of microtubule binding as the 64 KHC residues closest to the C-terminal are also required. They advanced a model in which the heptad repeats of KLC induce an interaction between the C-terminal tail and hydrolytic heads of KHC that prevents microtubule-binding. Once crystallographic analysis was possible, Kaan et al. (2011) could identify the components involved in autoinhibition and advanced a "double lockdown model" according to which the tail region of folded kinesin cross-links its ATP hydrolyzing heads resulting in a non-motile structure that inhibits ADP release.

Recognizing that kinesins inhibit themselves also pointed kinesin researchers to another new line of inquiry: determining what releases kinesin from autoinhibition. This led to the discovery of the first molecule that couples kinesin to cargo, Sunday Driver (SYD). Bowman et al. (2000) found it in the course of investigating the *Drosophila syd* mutant that exhibited the same defective transport phenotype as was produced by deletion of a subunit of kinesin itself. To explain its role, the researchers drew on contemporaneous research by Ito et al. (1999) and Kelkar, Gupta, Dickens, and Davis (2000) that revealed that SYD acts as a scaffolding protein in the MAPK/JNK signaling pathway that regulates cell functions such as autophagy. A scaffolding protein provides a structure along which proteins can be spatially organized so that they can easily interact with other proteins involved in the same process. Drawing on this framing,

[41] More recently Kelliher et al. (2018) have drawn on findings that kinesins bind to receptors on the Golgi apparatus to advance a new hypothesis that a function of kinesin autoinhibition is to maintain Golgi outposts in dendrites.

Bowman et al. proposed that SYD provides a scaffold that forces kinesin out of its autoinhibitory state and so enables it to bind cargo (in this case a vesicle) and begin to traverse a microtubule (Figure 10).

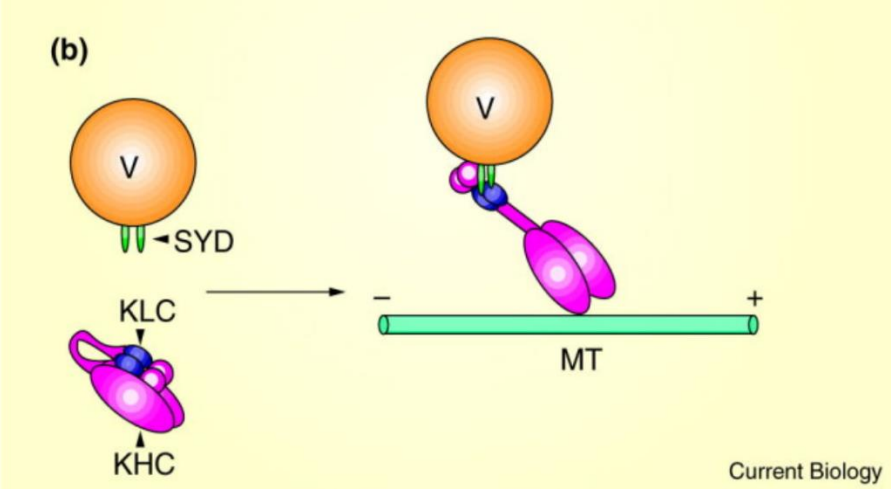

Figure 4.10: SYD linking kinesin to vesicular cargo. Reproduced with permission from Elsevier. Hays and Li (2001).

The findings about SYD were soon generalized. SYD is one of three JNK interacting proteins (JIPs). Research on the other two (JIP1 and JIP2) provided compelling evidence that they facilitate binding to other cargo when bound to a further membrane-associated protein, ApoER2 (Verhey & Rapoport, 2001). Drawing on their own and other research (Byrd et al., 2001), Verhey and Rapoport advanced a schema on which kinesin figures in the JNK-pathway. First, the cargo binding protein binds to the motor which releases it from autoinhibition, binds the motor to cargo and provides a scaffold for intracellular signaling kinases in the pathway. Next, the complex is transported to the nerve terminal where the cargo fuses with the plasma membrane (step 1 in Figure 11), binds its extra-cellular ligand (step 2), phosphorylates a

signaling kinase (step 3), and releases the kinesin (step 4) which resumes its autoinhibited configuration and diffuses (or is itself transported) through the cell.

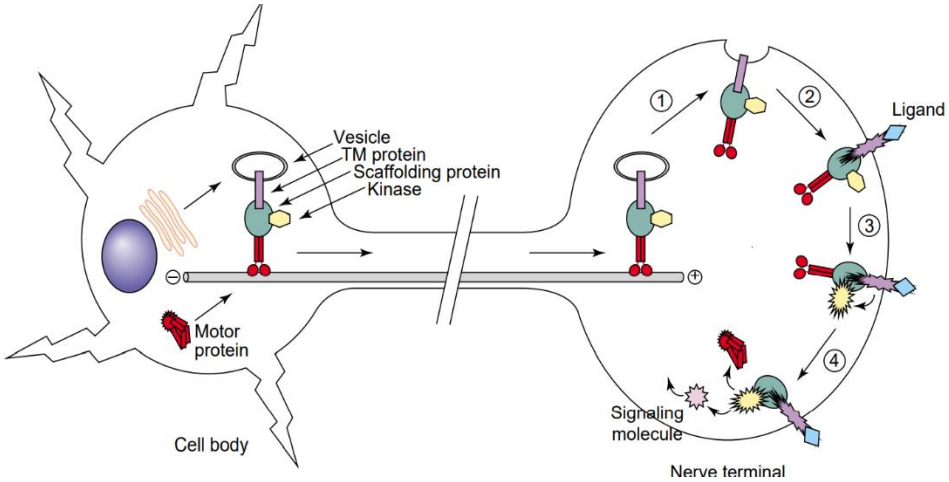

Figure 4.11: Cargo releases kinesin from autoinhibition in the cell body. Reproduced with permission from Elsevier. Verhey and Rapoport (2001).

Situating kinesin in this larger activity of transporting signaling molecules provides a different perspective on kinesin. It is not just a motor that generates movement from ATP but an entity whose operation is controlled by other entities in its environment. This is facilitated by cargo binding proteins. They determine which phenomenon a kinesin is to exhibit—autoinhibition or active transport. This perspective motivated much additional research during the past decade that has resulted in identifying additional cargo-binding proteins and additional means by which kinesin is released from autoinhibition (Lin & Sheng, 2015) and its transport activities regulated (Sirajuddin, Rice, & Vale, 2014).

In the kinesin case, soon after it was identified as the motor driving anterograde transport, investigation of key features of the assay used to demonstrate kinesin motility compelled researchers to associate a second phenomenon—autoinhibition—to the mechanism. This led researchers not just to focus on what was required to release kinesin from autoinhibition so that it

would produce motility but to situate kinesin in a larger context in which its activity is controlled by cargo binding proteins. These controlling cargo-binding proteins determined when kinesin was released from its autoinhibited state and transported cargo. In this case, the recognition that kinesins instantiated the design principle of autoinhibition initiated research into the entities that released it from autoinhibition and thereby regulated its activity. This is not the only trajectory research can take, however. Research on dynein reveals a different trajectory.

Section 5: Discovering how Cytoplasmic Dynein is Controlled

While developing procedures to reconstitute dynein motility *in vitro*, researchers came to recognize that other molecules had to be added to their preparation in order for dynein to generate movement. Researchers immediately conceptualized these additional molecules as controlling or regulating dynein's behavior. It took twenty-five years, however, for researchers to recognize that the molecules were, specifically, releasing the motor from autoinhibition. We examine how this research proceeded and reflect on why these additional components were considered control elements rather than simply parts of the mechanism for transport. We conclude this section by considering how this research culminated in the understanding that dynein produces a second phenomenon, autoinhibition, when these regulatory components are not present.

After developing an assay in which they could demonstrate retrograde movement along microtubules, Schroer, Steuer, and Sheetz (1989) tried to reconstitute dynein-driven motility using purified dynein. They found that purifying dynein and adding it back to a preparation of microtubules did generate movement, but much slower than in their initial preparation. The researchers concluded that some factor or factors other than dynein was required to generate

normal movement. Gill et al. (1991) showed that normal dynein movement could be restored by adding a large protein complex that they isolated from the original preparation and named *dynactin* (*dynein activator*). Moreover, Gill et al. demonstrated that when they removed it completely from a dynein preparation (the initial purification of dynein was only partial), motility was totally suppressed. Dynactin, they concluded, was required for dynein to generate retrograde transport.

The discovery of dynactin initiated an inquiry into how it interacts with dynein. One hypothesis stemmed from Gill et al.'s determination that the gene *dynactin* exhibited 50% sequence identity to the Drosophila *Glued* gene. Subsequent electron microcopy studies showed that the shared sequence corresponded to a $p150^{Glued}$ dimer that forms an arm (shown in Figure 12) that binds to both dynein's intermediate chain and the microtubule (Waterman-Storer, Karki, & Holzbaur, 1995). The significance of dynactin binding to the microtubule proved controversial. Since without dynactin, dynein could not maintain motility over long distances, King and Schroer (2000) proposed that the arm provided an additional contact that could keep dynein on the microtubule. However, Kardon, Reck-Peterson, and Vale (2009) demonstrated that if, in yeast, they rendered dynactin's arm unable to bind the microtubule, processivity still increased over preparations without dynactin. More recently Ayloo et al. (2014) have argued for important differences between yeast and mammalian dynein. They advance evidence that, in mammals, dynactin often binds to the microtubule before dynein, recruits dynein to it, and keeps dynein tethered to the microtubule (sometimes braking dynein's movement). They argue that these activities are essential for dynein to transport small cargoes, which employ only a few dyneins, and in regions of the cell in which there are few microtubules.

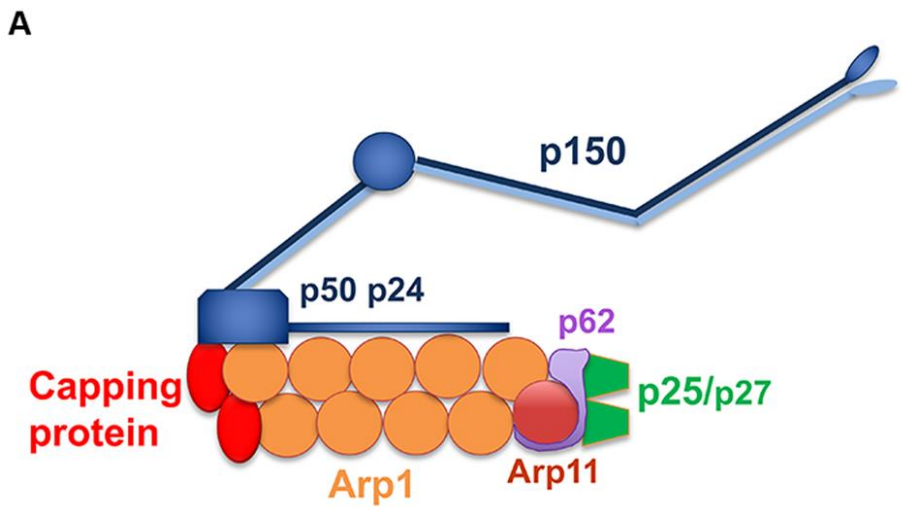

Figure 4.12: A schematic representation of the structure of dynactin. Reproduced under Creative Commons License CC BY from J. Zhang, Qiu, and Xiang (2018).

In spite of finding dynactin to be necessary to produce movement *in vitro*, the investigators did not simply treat it as an additional part of the mechanism for retrograde transport. Rather, they construed dynactin as *regulating* or *controlling* dynein which they continued to view as having a distinctive status, namely, the motor that drives the motion by transforming ATP into mechanical motion. In other words, they viewed the motor as the mechanism for transport and dynactin as regulating this mechanism. However, unlike in the kinesin case in which researchers already understood the motor as capable of inhibiting itself and, thus, understood the binding partners as releasing kinesin from its autoinhibited state, dynein researchers did not yet understand dynein to be capable of autoinhibition. Thus, prior to the discovery of dynein autoinhibition, dynactin was viewed as regulating dynein but not specifically by releasing it from autoinhibition.

Dynactin was just the first additional component that researchers discovered was required for dynein to produce retrograde motion. Researchers soon discovered that dynactin on its own does not tend to bind to dynein and when it does, the resulting dimer is unstable. Swan, Nguyen,

and Suter (1999) found that, in *Drosophila*, Bicaudal D[42] (BicD; in mammals BicD has two homologues, BicD1 and BicD2) promoted their binding. Hoogenraad et al. (2001) showed that BicD proteins form a complex with dynein and dynactin and Rab6, a small GTPase situated on membranes of vesicles synthesized in the Golgi apparatus. McKenney, Huynh, Tanenbaum, Bhabha, and Vale (2014) revealed that BicD2 provides a rigid structure to which both dynein and dynactin bind (Figure 13). Researchers responded to these findings as they had to dynactin—they did not treat BicD as a component of the mechanism for retrograde transport but as acting to regulate its activity.

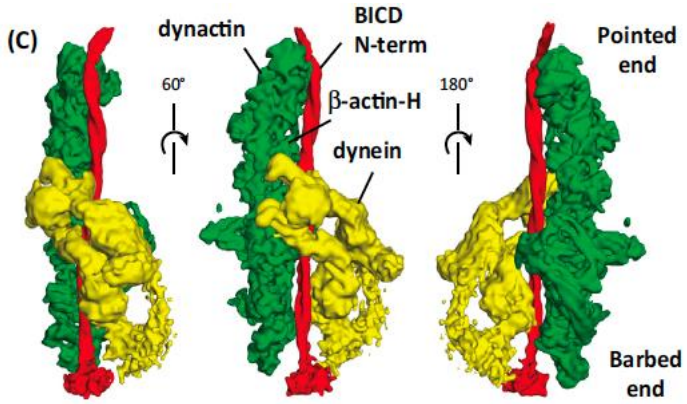

Figure 4.13: Role of BicD in generating a bond between dynein and dynactin. Reproduced with permission from Elsevier. Hoogenraad and Akhmanova (2016).

The fact that Rab6, a protein on the Golgi apparatus, is part of the complex that forms with dynactin, dynein, and BicD, pointed to a more specific role for BicD—recruiting dynein to an organelle requiring transport. Since vesicles produced in the Golgi apparatus are just one type of cargo transported by dynein, researchers searched for other agents that enable other cargos to bind to dynein. To date, they have identified several and the cargos to which they bind: Rab11-

[42] The protein was so named as it was first identified in a *Drosophila* mutant in which the anterior segments of the embryo become a set of second posterior segments.

FIP3 binds recycling endosomes, Hook3 binds secretory vesicles, and Spindly binds kinetochore (Canty & Yildiz, 2020).

From this, researchers concluded that dynactin and BicD (or another cargo-binding protein) are required to generate retrograde transport and act by controlling dynein's operation. This raised the question of what dynein does when it is not generating retrograde transport. An early micrograph by Amos (1989) had shown dynein in a conformation in which its "two heads fused together, forming a dimeric globular particle with two separate tails" (a conformation Amos named *phi* for its shape). This finding, however, was largely neglected until Torisawa et al. (2014) drew attention to it and identified the phi-conformation as an autoinhibited state in which dynein's two heads are stacked with their C-terminal sides facing each other and their stalks crossed. In this configuration the microtubule binding domains are facing in opposite directions, enabling only one of them to bind a microtubule. This makes processive movement impossible (Figure 14). When ATP is available, dynein in the phi conformation can bind and release from the microtubule, but this merely leads to dynein diffusing along the microtubule with a slight bias towards the minus end. Torisawa et al. also found that if they forced the two heads apart by inserting a rigid rod (emulating the effect of BicD), dynein movement became directed and processive. Given the role of cargo-binding proteins in recruiting BicD to dynein, they proposed this control process ensured that dynein only assumed a structure in which it could act as a motor when cargo was in need of transport.

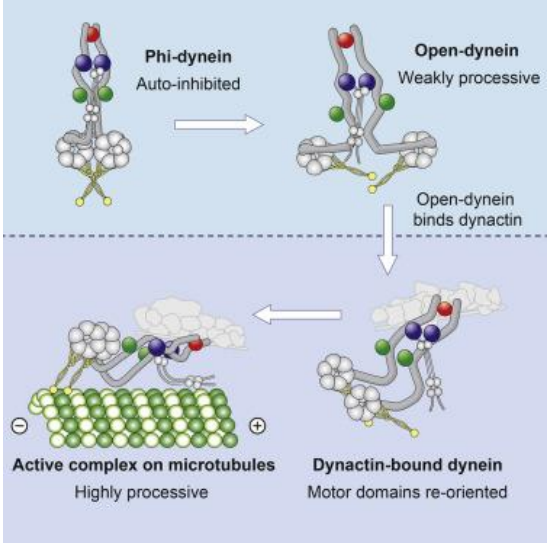

Figure 4.14: Transformation of dynein from phi-particle conformation to binding with dynactin and becoming an active motor. Reproduced under Creative Commons License (CC BY) from K. Zhang et al. (2017).

Recognition that dynein instantiates the design principle of autoinhibition has led to an explosion of proposals as to how dynactin and BicD figure in autoinhibition release. The use of a rod by Torisawa et al., for example, suggested that this is the role played by the Arp1 component of dynactin (this proposal received further support from an EM study by K. Zhang et al., 2017). As we noted, dynein and dynactin on their own do not bind and BicD has been viewed as playing an activating role by providing a rigid structure along which both dynactin and dynein can bind. McKenney (2018) proposes that binding to dynactin and BicD breaks the symmetry of the autoinhibited dynein. Researchers have developed similar accounts of the mechanical action of other cargo adaptors (Reck-Peterson, Redwine, Vale, & Carter, 2018; Olenick & Holzbaur, 2019).

The research on dynein followed a different trajectory as that on kinesin, but both resulted in a major change in perspective from one in which the motors were just understood as engaging in motility to one in which they were normally autoinhibited and only produced

motility when cargo needed to be transported. In the case of dynein, the shift started with the recognition that additional elements were needed for dynein to generate motility and the treatment of these as control elements, necessary for dynein to generate processive movement. It was not until 2014 that researchers came to see dynein as capable of adopting a functionally distinctive state and autoinhibiting when these control elements were not active. Once its capacity to autoinhibit was recognized, it was further recognized that the specific roles of dynactin and BicD were to release the motor from its autoinhibited state. Thus, in the case of dynein as well as kinesin, researchers came to adopt a perspective on the motor from which the motors were seen as performing two distinctive functions—movement and autoinhibition—under different conditions of control.

Section 6: Implications of Discovery of Autoinhibition for Philosophical Accounts of Mechanisms

Our account of the discovery that kinesin and dynein instantiate the design principle of autoinhibition—molecular mechanisms inhibiting themselves and only producing the phenomenon of motility when released by a control process—has significant implications for standard accounts of mechanistic explanation (Machamer et al., 2000; Bechtel & Abrahamsen, 2005). We begin with implications for the characterization of the discovery process as beginning with characterizing a phenomenon and then discovering the mechanism responsible for it. The discovery of kinesin and dynein autoinhibition followed the reverse path—starting with the mechanisms and determining that, in addition to motility, they exhibit autoinhibition. This also brings into question the common assumption of a one-to-one mapping of a phenomenon unto a mechanism. We then turn to implications of the fact that mechanisms are subject to control for

standard accounts of the constituency of mechanisms. We argue that an alternative conception of mechanism as consisting of constraints that direct the flow of free energy provides a better understanding of how mechanisms are subject to control.

Standard accounts of mechanism discovery embrace a *phenomenon-first* approach to inquiry, as described by Illari and Williamson (2012, p. 123):

> All mechanistic explanations begin with (a) the identification of a phenomenon or some phenomena to be explained, (b) proceed by decomposition into the entities and activities relevant to the phenomenon, and (c) give the organization of the entities and activities by which they produce the phenomenon.

On this view, the characterization of the phenomenon is the reference point for identifying the mechanism. The initial research on both kinesin and dynein adhered to this strategy, seeking mechanisms *for* active transport and, subsequently, *for* the stepping patterns of the motor mechanisms. But the research leading to the discovery of autoinhibition departed from this approach, instead starting with the mechanisms and developing from investigations of the mechanisms a characterization of a second phenomenon for which it was responsible: autoinhibition. In this process, the mechanisms served as the reference points for discovering the phenomena.

There is precedent in the mechanist literature for identifying phenomena based on an account of a mechanism. In her discussion of "phenomenon reconstitution," Kronfeldner (2015) describes how researchers can pick out a particular "causal factor," experiment and collect data on it, and then treat it as explanatory with respect to a different phenomenon than that which researchers were initially investigating. Bechtel and Richardson (1993/2010) tell a similar story in their discussion of the "Mendelian trait" which was initially understood as a macroscopic

phenotypic trait (e.g., eye color). Finding that patterns of phenotypic inheritance cannot be explained in terms of single genes, scientists re-identified the phenotypic trait with something that could be explained in terms of single genes—enzyme activity. Thus, the phenomenon to be explained in terms of single genes was "reconstituted" from the phenotypic trait to enzyme activity.

Thus, while the simple narrative of mechanistic inquiry takes it to start with the identification of a particular phenomenon and to proceed by seeking the underlying mechanism, it is recognized that, in the iterative process of mechanistic investigation, mechanisms themselves can take the lead with researchers holding them fixed to scaffold inquiry while the phenomena to be explained undergo renovation. Accordingly, after presenting the phenomenon-first account cited above, Illari and Williamson go on to characterize a more nuanced process:

> Mechanisms are individuated by their phenomena, and phenomena are also individuated by their mechanisms. This is not circular, because it happens iteratively over time. At the beginning, a mechanism is not needed to individuate a phenomenon, but the characterisation of the phenomenon may be further refined when a mechanism or mechanisms are discovered" (124).

Even on this more nuanced view, however, the process ends with a single phenomenon explained in terms of a single mechanism (or "causal factor"). In the research we described, however, the conclusion was not a single reconstituted phenomenon but the recognition that, by design, the same mechanism was responsible for two different phenomena. This is not a trivial modification of standard mechanistic accounts according to which mechanisms are individuated by the phenomena they explain. Following Glennan's (1996) assertion, "One cannot even identify a mechanism without saying what it is that the mechanism does," the principle that the

identity of a mechanism is tied to the phenomenon it explains has been called Glennan's Law.[43] The identification of autoinhibition as a second phenomenon associated with molecular motors would be a violation of this principle. In light of the fact that research often does proceed from characterization of a phenomenon to the identification of a mechanism, we suggest that the one phenomenon-one mechanism principle might better be treated as a heuristic that can productively guide research but can also be expected to fail, especially as research proceeds.

Recognizing that the same mechanism can produce two incompatible phenomena, such as motility and autoinhibition, raises a further question: what determines which phenomena it produces on a given occasion? In the cases of kinesin and dynein, it was cargo binding proteins that, by binding to the motors, induce a change in conformation that releases them from autoinhibition and enables them to adopt a conformation in which they can bind microtubules, bind ATP, and walk along the microtubule. This presents another challenge. Even before dynein researchers identified it as inhibiting itself, researchers had identified the need for dynactin and an agent like BicD in order for it to generate motility. We noted that researchers did not treat these agents as parts of the mechanism but as ones that controlled the mechanism. But on a common view about the identity of mechanisms, these agents would be identified as components of the mechanism. Craver (2007a; see also Craver and Kaplan 2020), for example, advances a constitutive relevance account for identifying the components of a mechanism. He employs the criterion of mutual manipulability—any factor whose manipulation can alter the phenomenon in terms of which the mechanism is identified and that is altered when the phenomenon is altered counts as part of the mechanism. On such a criterion, dynactin, BicD, etc., all count as

[43] Not all mechanists have ascribed to it. In their definition of a mechanism, Bechtel and Abrahamsen (2005, p. 423) allow that a mechanism may be "responsible for one or more phenomena."

constituents of the mechanism responsible for retrograde motility. Again, though, this is not how researchers understood these additional required elements.

If one adopts the mutual manipulability criterion of constitutive relevance, one can maintain Glennan's law and avoid attributing more than one phenomenon to a mechanism—from such a perspective, different mechanisms are responsible for motility and autoinhibition. The history that we analyzed in sections 4 and 5 would be the history of discovering a new phenomenon for which a separate mechanism was responsible. We resist this proposal. First, this is not how the scientists characterized their accomplishment. They understood themselves to have determined that the mechanism responsible for motility inhibited itself when appropriate control processes did not operate on it. Second, there is an important distinction to be made between mechanisms responsible for specific phenomena and control processes (mechanisms) that operate on them. Control is important not just for mechanisms that autoinhibit. Under such rubrics as *cell signaling*, biologists are increasingly focusing on how mechanisms within living organisms are controlled.

If one rejects mutual manipulability as the criterion for identifying constituents of mechanisms, one needs an alternative criterion. Such an alternative is found in the proposal by Winning and Bechtel (2018) to characterize mechanisms not as collections of entities and activities responsible for a phenomenon, but as entities that constrain flows of free energy so as to perform the work needed to produce the phenomenon to be explained. Free energy and work have not featured in new mechanist accounts. To account for the active nature of mechanisms, Machamer et al. (2000) treat activities as constituting a primitive category that does not require explanation. An alternative is to follow physics and treat free energy as required for activity. Without free energy, mechanisms are inert. The biologists investigating motility recognized

this—they assumed the source of free energy for motility was provided by ATP and accordingly looked for an ATPase that interacted with microtubules. Kinesins and dyneins are both ATPases—by hydrolyzing ATP they release free energy which is then constrained to produce the movements within these proteins (Bechtel & Bollhagen, 2021).

Adopting the conception of mechanisms as sets of constraints that direct the flow of free energy, one can differentiate mechanisms from other processes that control them. The mechanism consists of the constraints that determine the work that is done from a given source of free energy. Controlling the mechanism also requires the performance of work—the mechanism is controlled by altering constraints within it. In the cases of kinesin and dynein, the cargo binding proteins perform the work of releasing the motors from autoinhibition. To perform this work, the control processes draw on their own sources of free energy and must constrain it appropriately. We cannot develop a full characterization of control processes here (for further development, see Bich & Bechtel, 2022a, 2022b); what is important for our purposes is that by attending to how mechanisms constrain free energy in the performance of work, one can distinguish mechanisms from other processes that exercise control over them. This revised account of mechanisms enables us to make sense of the researchers' distinction between kinesins and dyneins and the processes that exercised control over them. Specifically, it enables us to understand why dynein researchers did not count dynactin, BicD, etc. as merely further parts of the mechanism for motility but, rather, as parts of mechanisms controlling dynein. Moreover, one can also understand how the same mechanism can be responsible for different phenomena—different phenomena are produced when the constraints within the mechanism direct free energy differently. In the case of kinesin and dynein, they autoinhibit rather than producing motility

when constraints within them prevent hydrolysis of ATP. When control processes operate on them, these constraints are altered and the motors hydrolyze ATP and generate motility.

Conclusion

Biologists are finding that many molecular mechanisms inhibit themselves. We have argued that autoinhibition constitutes a design principle—an abstractly characterized pattern of organization that explains phenomena across a wide range of cases—and that accounts of mechanisms that maintain mechanisms to be individuated by the (single) phenomenon they explain struggle to accommodate mechanisms that instantiate this design. We suggest that the account of biological mechanisms developed in Winning and Bechtel (2018) provides a positive alternative.

Mechanisms exhibiting this design principle inhibit their own activity through *intra*molecular interactions which prevent them from generating the phenomenon characteristic of their active states. *Inter*molecular interactions between the mechanism and binding partners release the mechanisms from autoinhibition, enabling them to produce that phenomenon. Focusing on two molecular mechanisms, kinesin and dynein, we analyzed how researchers discovered that they autoinhibit. We showed that different paths were followed in the two cases. In the case of kinesin, researchers quickly recognized that the experimental protocol they deployed to investigate kinesin motility acted to release kinesin from a conformation in which it inhibited itself. Research then turned to what processes act on kinesin in living cells to control it by releasing it from autoinhibition. In the case of dynein, researchers early on recognized a that a variety of other entities were needed for dynein to produce motion *in vitro* but, contrary to what the mutual manipulability criterion would imply, researchers did not consider them part of the

mechanism for retrograde axonal transport. Rather, they construed them as controlling the mechanism. Only much later did researchers recognize that such control was needed to release dynein from, specifically, autoinhibition. In both cases, the shift in perspective involved in the discovery—the shift from understanding the proteins as "denatured" or otherwise simply not producing motion in *in vitro* assays to understanding them as implementing an autoinhibitory design—prompted researchers to discover broader processes in the cell which functioned to release the motors from their autoinhibited state. In the end, researchers arrived at a framework in which molecular motors exhibit the design principle of autoinhibition and only produce motility when acted on by control processes.

Autoinhibition is widely implemented in biology. In fact, as Pufall and Graves point out, there are likely numerous undiscovered instantiations of this design principle.[44] Thus, a philosophical account of biological mechanisms needs to be able to accommodate this important organizational pattern. That autoinhibitory mechanisms exhibit two phenomena—e.g., autoinhibition and motility—with control processes determining which they exhibit on a given occasion does not fit well with the standard philosophical accounts of mechanisms. Accordingly, we provide a revised philosophical account of mechanisms that distinguishes control processes from the operations of the controlled mechanism and recognizes that one mechanism can exhibit multiple phenomena. This revised account of mechanisms is well-suited to understand both the various biological mechanisms that implement autoinhibition as a design principle and the process involved in their discovery.

[44] See fn. 3.

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Chapter 4, in full, is a reprint of the material as it appears in Bollhagen, A., & Bechtel, W. (2022). Discovering autoinhibition as a design principle for the control of biological mechanisms. *Studies in History and Philosophy of Science*, *95*, 145-157. The dissertation author was the primary investigator and author of this paper.

Chapter 5: Process or Mechanism? Implications of Brownian Ratchet Accounts of Molecular Motor Activity

Section 1: Introduction

Recently a number of philosophers have advanced a processual framing of biology (for a useful overview, see the papers collected in Nicholson & Dupré, 2018) as a replacement for the mechanistic approach to explaining biological phenomena that has dominated biological research for the past two-hundred years and that has been characterized by new mechanists in the philosophy of science (Machamer, Darden, & Craver, 2000; Bechtel & Abrahamsen, 2005; Glennan, 2017). Just as philosophical accounts of mechanistic explanation draw inspiration from specific explanations offered by biologists (Bechtel & Richardson, 1993/2010; Craver & Darden, 2013), processual theorists often ground their account in examples from biology. Nicholson (2019, 2020), in particular, has promoted a process account by arguing against mechanistic accounts of molecular motors—proteins such as myosin, kinesin, and dynein that transport cargo along cytoskeletal filaments (microtubules or actin filaments) in cells. The mechanistic accounts he criticizes invoke a powerstroke in which free energy, liberated by hydrolysis of ATP, is used to change the conformation of the protein to induce movement. For an alternative, Nicholson draws upon accounts that appeal to Brownian motion due to thermal energy to explain the forward movement. A mechanistic process such as a powerstroke, on his interpretation of the science, could not explain the force and motion generating activities of molecular motors. It also wrongly applies ideas from the macroscopic world to the nanoscale, at which Brownian motion predominates. For Nicholson, appealing to a mechanistic process such as a powerstroke is a mistake rooted in a faulty analogy—the machine analogy—between human-made macroscale machines and the nanoscale processes operative in biological organisms.

To assess this specific challenge to mechanistic explanations, we will examine whether Brownian motion accounts of molecular motors provide non-mechanistic alternatives to powerstroke accounts. We will argue that they do not. On the one hand, to derive directional motion from random thermal motion, proponents of Brownian motion accounts of molecular motors follow Feynman in appealing to devices such as ratchets. Although they characterize ratchets only in metaphorical and mathematical terms and so avoid positing any specific physical molecular structure to them, ratchets must be realized in the physical world as mechanical devices. Accordingly, the explanation of how directional motion is derived from Brownian motion requires a mechanism. On the other hand, while Brownian motion does play an important role in the movement of molecular motors and chemists have constructed synthetic motors relying on it for their locomotion (movement along the cytoskeleton), according to most theorists, it does not suffice to account for the locomotive movement of myosin, kinesin, or dynein. Many theorists who appeal to Brownian ratchets to explain the activities of these motors integrate them with a mechanism deriving force from a powerstroke. Accordingly, the scientific proponents of Brownian ratchet accounts in different ways embrace mechanistic accounts in which free energy is used to generate unidirectional motion.

A couple of qualifications are required before we turn to assessing the alternative explanations of how molecular motors generate motion. First, Nicholson presents his discussion of molecular motors in the context of a broader challenge to mechanism. It is important to recognize respects in which the characterization of mechanism adopted by those who advance a mechanistic framing of explanation in biology differs from the one Nicholson criticizes. In adopting the term *mechanism*, both biologists and new mechanists in philosophy of science (Machamer et al., 2000; Bechtel & Abrahamsen, 2005; Glennan, 2017) advance an analogy with

human made machines. The key feature of the analogy is to appeal to the organized component entities and activities of a mechanism to explain a phenomenon. Invoking an analogy is compatible with recognizing differences between the items being compared. Nicholson often employs the term *mechanicism* and imputes to both biologists and new mechanists other features of the analogy that they do not embrace, such as that mechanisms are fixed entities like the machines in a factory. Both biologists and new mechanists recognize that biological mechanisms are made out of different types of materials than typical machines made by humans and as a result lack rigidity. Moreover, both recognize that biological mechanisms are dynamic (Bechtel & Abrahamsen, 2010; Brigandt, 2013). The dynamic nature of mechanisms extends to their construction and demise: many mechanisms are assembled on a just-as-needed basis, undergo changes as they operate, and are afterwards disassembled (Green et al., 2018). What is required to characterize them as mechanisms is that, at the timescale in which a phenomenon is generated, researchers can identify the mechanism, individuate its parts, and assign activities to them.

Second, Nicholson raises his objections to powerstroke models of molecular motors in the context of a broader critique of what he terms the Machine Conception of the Cell (sometimes Machine Conception of the Organism). We do not embrace treating cells or multicellular organisms as mechanisms, let alone machines. We view cells and multicellular organisms in much the same manner as Nicholson does—as far from equilibrium systems that maintain themselves by performing a host of activities (Moreno & Mossio, 2015). We view mechanisms as theoretical posits advanced to explain the activities of cells or organisms. How mechanisms as we understand them relate to cells or organisms is a further important issue that we will not address here (for a discussion of this issue, see Bich & Bechtel, 2022). Rather, our focus is restricted to the competing strategies for explaining the movement of molecular motors

and whether Brownian ratchet accounts constitute a genuinely non-mechanistic alternative to approaches that invoke a powerstroke.

Third, the principal difference between process accounts and mechanistic accounts is the role of entities in the later. Activities, as characterized by Machamer et al. (2000), correspond to processes in process accounts. Both Machamer et al. and process theorists treat activities or processes as metaphysically fundamental. Winning and Bechtel (2018), however, have dissented from Machamer et al.'s treatment of activities, arguing that activities in the natural world can themselves be explained in terms of two basic concepts of physics, Gibbs free energy and constraints. Applied to mechanisms, the basic idea is that mechanisms perform work when they appropriately constrain free energy, and that it is by altering which constraints are operative at a time that mechanisms are made to perform different work. This conception of mechanisms as involving constraints on the flow of free energy is directly applicable to powerstroke accounts of molecular motors—motors generate specific movements as a result of the molecular composition of the motor constraining the release of free energy.[45] Although Winning and Bechtel did not consider Brownian motion or thermal energy, the challenge for deriving work from it also involves imposing constraints (which in turn depends on constraining the flow of Gibbs free energy). This conception of mechanism also makes the comparison and contrast with process accounts clear—on mechanist accounts, energy does flow, to use the language advanced by the process theorists, but it is the specific constraints that are instantiated at a time that determine what work is done by the flow of energy. We return to this way of conceptualizing the disagreements between process theorists and mechanists in the concluding section.

[45] See Bechtel and Bollhagen (2021) who apply this conception of mechanism to molecular motors.

As the idea of a Brownian ratchet may be unfamiliar to many readers, we offer here a brief introduction that we will build upon in subsequent sections. The basic idea has its origins in Maxwell's (1871) thought experiment in which a demon enabled the segregation of initially equally distributed molecules with higher and lower kinetic energy into two adjoining containers by operating a trap door to allow molecules with high kinetic energy to pass into one of the containers. The demon, however, could only operate by drawing upon a source of free energy. The thought experiment was further developed by Feynman, who proposed a configuration in which a vane and a ratchet and pawl are at opposite ends of a fixed pole so that they rotate together, with the vane in one box and the ratchet and pawl in another (Figure 1). The teeth of the ratchet are asymmetric such that, on one side, the tooth extends perpendicularly from the circumference of the ratchet while, on the other side, it extends at a shallower angle. One might think that, like a macroscale ratchet, the wheel will only turn in the direction of the tooth with the shallower angle (the clockwise direction), resulting in the ratchet turning clockwise. However, in this microscale device, the pawl itself undergoes Brownian motion due to thermal forces in its box and therefore "bounces," allowing the pawl to slip over the tooth in the counterclockwise direction. Thus, if the temperature of the boxes is the same, the wheel will turn clockwise and counterclockwise with equal probability resulting in no net motion in either direction. However, if the temperature in T1 > T2, the thermal energy transmitted from the vane to the wheel will be greater than the thermal energy "bouncing" the pawl. This will, because of the asymmetry of the teeth, lead to net clockwise rotation and the load suspended between the boxes will lift. Free energy, as opposed to the thermal energy of Brownian motion, is required to maintain this temperature difference. Brownian ratchet accounts of the movement of molecular motors treat the motors as moving along actin or microtubules through Brownian motion, with the motor

molecules rectifying this process by biasing overall movement to occur in only one direction. As in the case envisaged by Feynman, rectification requires an application of free energy.

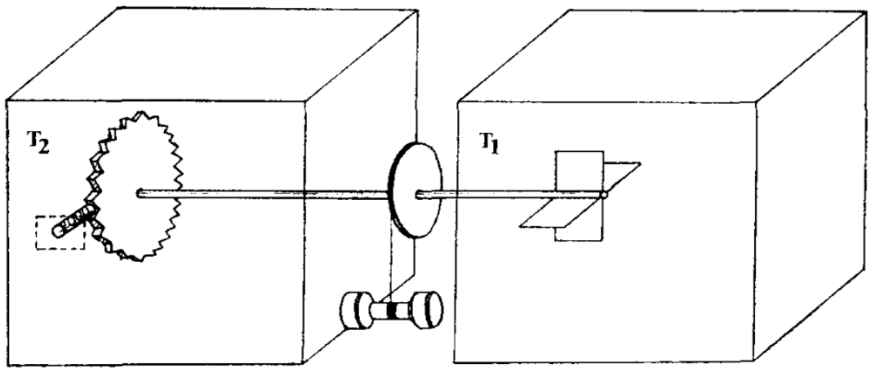

Figure 5.1: Representation of Feynman's ratchet from Vale and Oosawa (1990)

Modern research on molecular motors can be dated to two paper that appeared back-to-back in *Nature* in 1954 that showed that in muscle contraction, fibrils of actin and myosin moved past each other (A. F. Huxley & Niedergerke, 1954; H. E. Huxley & Hanson, 1954; the two Huxleys are not related). In the 1980s two other molecular motors, kinesin and cytoplasmic dynein, were discovered as moving along microtubules (Vale, Reese, & Sheetz, 1985; Vallee, Wall, Paschal, & Shpetner, 1988). Following their discovery, a great deal of research was devoted to explaining their motion, with some of the proposed models invoking Brownian ratchets, others powerstrokes. During this period, however, researchers were quite non-committal, often warning readers that the models were merely hypothetical. We begin in section 2 by examining two Brownian ratchet accounts that were advanced in this period by Andrew Huxley and Ronald Vale. We show that each advanced his account as advancing a hypothetical mechanism that provided a foundation for developing mathematical analyses of motor activity. As powerstroke accounts developed, both embraced them. Because the attitude they adopted

toward their ratchet models was non-committal to begin with, and because of their eventual acceptance of powerstroke accounts, neither Andrew Huxley nor Vale can be viewed as rejecting mechanistic analysis or manifesting principled opposition to powerstroke accounts. In fact, at this point in the history, powerstroke and ratchet models were not pitched against each other as opposed analyses of molecular motor activity in the way that they came to be later.

The ratchet accounts to which Nicholson appeals as alternatives to powerstroke accounts were developed after the development of robust powerstroke models in the 1990s and were explicitly offered to challenge the explanatory framework that appealed to powerstrokes. In section 3 we present Nicholson's characterization of Brownian motion models as well as those of Astumian, on whom he draws, and Baker, who offers an alternative account that provides insights to the basis for opposing powerstroke accounts. We show that both Astumian's and Baker's accounts are, nonetheless, mechanistic. In section 4 we turn to the account provided by other researchers invoked by Nicholson—Ait-Haddou and Herzog. We show that, while applying a ratchet conceptual framing and mathematical treatment similar to Astumian and Baker, Ait-Haddou and Herzog actually employ a powerstroke to generate locomotion of the motor molecules.

Section 2: Brownian Ratchet Models Advanced in the Absence of Powerstroke Models

We begin in this section by focusing on major early investigations into molecular motors in which researchers advanced Brownian-motion based accounts. What is notable about these scientists is that they were doing so without empirical evidence strongly favoring either Brownian motion or powerstroke models. Although researchers put forward alternative models, there was no "debate" between a "powerstroke camp" and a "ratchet camp," such as emerged

during the period we consider in subsequent sections. Although these mechanistic models were speculative, this does not mean that they played no substantive role in the investigations of these early researchers. As we will see, they functioned as what might be called "placeholder mechanisms." As such, they facilitated researchers in developing formal analyses that they could compare to equations formulated by Hill (1938) to describe the observed relationships between heat, energy, and force production in the contraction of muscle and by Feynman in his analysis of his Brownian ratchet. This formal work was not seen by as an end in itself. Rather, both Andrew Huxley and Vale and Oosawa saw it as facilitating further experimentation using techniques of mechanistic discovery. Thus, for these early researchers, the Brownian ratchet account was not seen as non-mechanistic but as a particular mechanistic account of energy transduction in molecular motor systems.

The pioneering papers by Andrew Huxley and Niedergerke (1954) and by Hugh Huxley and Hanson (1954) that revealed that myosin and actin filaments slide along each other so as to shorten the overall length of the muscle fiber did not themselves advance an explanation for this movement. Both Huxleys directed their subsequent research to developing such explanations. Andrew Huxley (1957) is credited with presenting the first mechanistic description of the process. Several decades earlier, Hill (1938) had advanced a phenomenal model relating the shortening of muscle, heat production, and the total energy liberated during contraction. Hill himself went beyond the phenomenal model to suggest that there are discrete "active sites" on the proteins that operate in a cyclical manner to drive contraction.[46] Inspired by this work, Andrew Huxley hypothetically characterized those sites as each involving an elastic element (M) which would oscillate back and forth due to thermal agitations (Figure 2). A rate parameter

[46] This was opposed to a received model on which the contraction involved a continuous folding or coiling of the protein filaments. See A. F. Huxley (2000a).

characterizes the reaction that binds M to the site on actin (A). It increases with distance x while another rate parameter that characterizes the ATP-involving reaction that releases M from A increases when x decreases. Once M binds to A, the thermal energy stored in the stretched spring can perform work on the filament. As the actin filament slides to the left, the distance x shortens and M releases. Importantly, the role of energy contributed by ATP is to break the link between actin and myosin while thermal energy stored in the springs drives the relative sliding (locomotion) of the filaments.

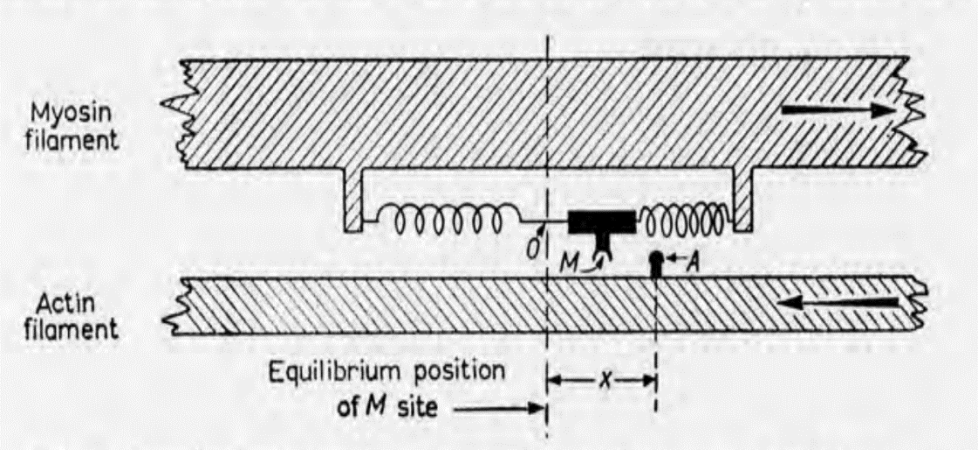

Figure 5.2: Andrew Huxley's (1957) Brownian ratchet account of the movement of myosin along actin. Thermal agitations drives M, an elastic element toward *A*, a binding site.

While Andrew Huxley presents himself as advancing a hypothetical mechanism (springs are mechanistic components), he is clear-eyed that it lacked experimental support. As he puts it, any such mechanical scheme ". . . at the present time, must necessarily be highly speculative, but may nevertheless be helpful both in showing how far it may be possible to go in explaining the known behaviour of muscle, and also — more important — in suggesting experiments which may exclude particular hypotheses or groups of hypotheses" (p. 280).

Acknowledging these limitations, Andrew Huxley formulated equations describing this speculative mechanical model mathematically and showed that he could recover, in his formal analysis, the relations specified in Hill's phenomenal model developed decades earlier. After reporting this important mathematical result, he again reminds readers that the physical speculations ought to be taken with a grain of salt:

> it must be emphasized that the agreement which has been achieved with some aspects of the known behaviour of muscle is not to be regarded as grounds for accepting the scheme which has been put forward. There is little doubt that equally good agreement could be reached on very different sets of assumptions, all equally consistent with the structural, physical, and chemical data to which this set has been fitted. The agreement does however show that this type of mechanism deserves to be seriously considered, and that it is worth looking for direct evidence of the side-pieces [Hill's "active sites"], and of the localization of enzymatic activity, which have been postulated (1957, p. 279).

In other words, the mechanically detailed model served for Andrew Huxley as a "placeholder" to give hypothetical physical meaning to the "active sites" implied by Hill's earlier work and to serve as a target for his mathematical analysis. While showing that, mathematically, such a model squares with Hill's earlier analysis, he emphasized that success on this score should not be taken as evidence for the reality of these physical posits because different sets of physical assumptions could serve equally well (A. F. Huxley, 2000b; see also Noble, 2022).[47] Importantly, he saw his formal analysis not as a satisfactory explanation in its own right, but merely as a means to facilitate further mechanistic inquiry.

[47] Interestingly, this is precisely how Andrew Huxley characterizes the status of the differential equations he and Hodgkin formulated to describe the action potential in neurons for which they received the Nobel Prize (Hodgkin & Huxley, 1952). See (Bickle, 2023). We take the consilience with Bickle's reading as evidence that our analyses are tracking an important feature of Huxley's research practices.

Hugh Huxley adopted a different approach, creating detailed x-ray images of myosin and actin fibrils. In these he localized Hill's "active sites" in what he identified in his images as "crossbridges" between actin and myosin filaments (H. E. Huxley, 1958, 1963). On the basis of the x-ray images as well as electron micrographs and biochemical data, Hugh Huxley (1969) advanced a mechanistic proposal on which, while attached to actin, the head component of myosin undergoes a large conformational change that alters its angle relative to actin and thereby imposes a force driving the movement of actin. As opposed to Andrew Huxley's model, on which the energy from Brownian motion is stored in an elastic "spring" outside the myosin head, Hugh Huxley associated ATP hydrolysis with a conformational change of the crossbridge which, in turn, performs work on the actin filament and drives relative filament sliding. This became known as the "swinging cross-bridge" hypothesis.

Shortly after Hugh Huxley advanced his hypothesis, two biochemists, Lymn and Taylor (1971), formulated a kinetic model mapping the stages of ATP hydrolysis to stages in the binding and movement of the myosin crossbridges: as shown in Figure 3, myosin detaches from actin as it binds ATP (1) and as it hydrolyzes ATP it changes its orientation (2). It then binds again to actin (3) and as it expels the products of ATP hydrolysis (ADP and Pi), it executes what Lymn and Taylor called a "drive stroke" corresponding to Hugh Huxley's large conformational change in the myosin crossbridges (4).

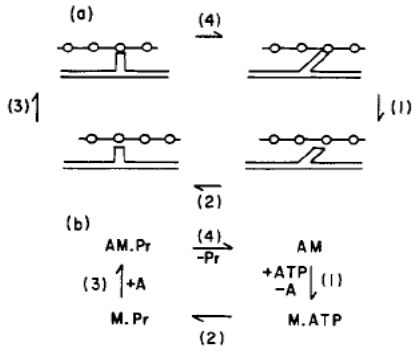

Figure 5.3: Lymn and Taylor's kinetic model with corresponding biochemical states. From Lymn and Taylor (1971)

Like Andrew Huxley, Lymn and Taylor warn readers not to overinterpret their model. While it might be natural to "suppose that either the binding or the splitting of ATP induces a configuration change which accounts for the movement of the free bridge [step 2 above]", they acknowledge "The kinetic scheme could also be made to fit the general features of the model of [Andrew] Huxley in which the movement of free bridges is due to thermal energy rather than interaction with substrate" (p. 4623). Thus, while Hugh Huxley's model associates the energy from ATP with a force-generating change in the conformation of myosin's head while bound to actin, Lymn and Taylor warn readers that the jury is still out and that a model like Andrew Huxley's is just as plausible given the available data.

With further research, Hugh Huxley's model had, by 1990, achieved the "stature of dogma in the view of college textbooks." Even so, several researchers noted that experimental evidence for a large conformational change in myosin crossbridges during force generation was lacking (Cooke, 1986; Goody & Holmes, 1983). In its absence, Vale and Oosawa (1990) formulated a "thermal ratchet" model that draws explicitly on Feynman's ratchet summarized above and shown in Figure 1. In it they propose a number of hypothetical mechanical models for myosin to draw on Brownian motion. Like Huxley, they compare their mathematical analyses of

these models with Hill's equations in an attempt to see how far analogizing actomyosin contractile systems with Feynman's ratchet can go toward recovering the relations between force, heat, energy, and velocity that Hill characterizes for contracting muscle.

Clockwise rotation of Feynman's ratchet occurs only with a temperature asymmetry: T1 > T2. In the muscle sarcomere, however, actin and myosin filaments are not isolated in chambers at different temperatures. Therefore, Vale and Oosawa argue, thermal energy alone is insufficient to explain the unidirectional motion of myosin motors: "without the input of chemical energy, thermal energy would displace the motor to adjacent polymer subunits in either direction with equal probability, as is true of the Feynman Ratchet when there is no temperature difference between the ratchet and the pawl" (p. 103). To preserve the analogy with Feynman's ratchet, Vale and Oosawa suppose that energy from ATP might induce an apparent "temperature" difference across the myosin and actin filaments sufficient to drive unidirectional motion by inducing either myosin heads or actin monomers to fluctuate more quickly than the other. Since both actin and myosin are proteins and since "most proteins undergo vibrational motions" they argue that "it is . . . reasonable to imagine that both the pawl and the ratchet contain spring elements that vibrate by thermal energy" (p. 107); see Figure 4. This represents a departure from Andrew Huxley's mechanical model which accounts for directionality by appeal to an asymmetry in the rate constants of myosin binding vs. release reactions which are, in turn, a function of the distance x in Figure 2. In spite of these differences, their model, like Andrew Huxley's, appeals to mechanical, "spring-like" elements, which constrain energy enabling the mechanism to do work.

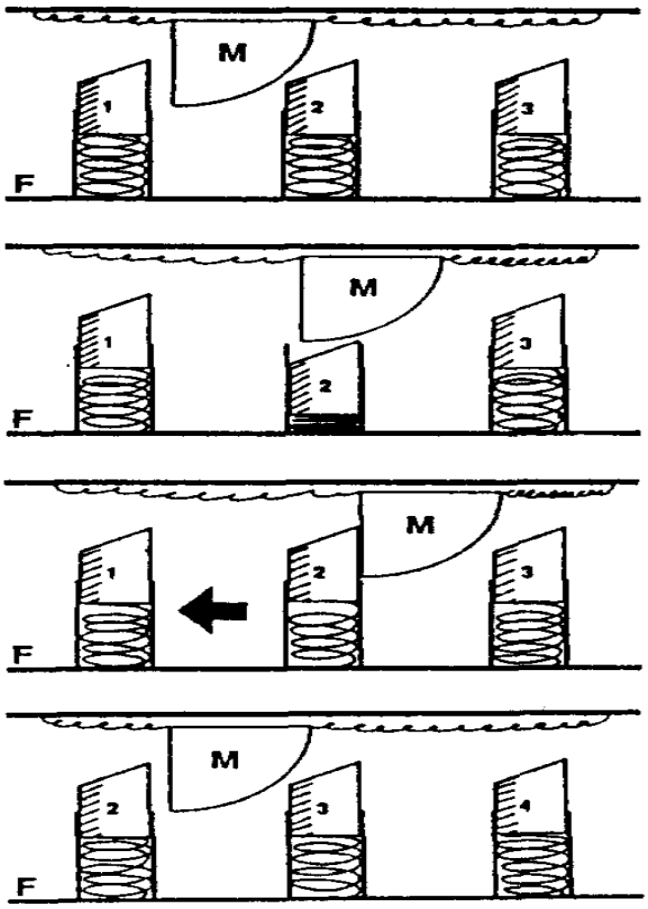

Figure 5.4: Vale and Oosawa' (1991) Brownian motion model with spring-like elements

What is important to note, for our purposes, is that both Andrew Huxley's and Vale and Oosawa's speculative Brownian ratchet models are, like Feynman's own model, explicitly mechanical.[48] While Nicholson cites the putative difference between macroscale machines and molecular biological systems as reason to think no useful analogy between the two kinds of systems can be drawn, these researchers explicitly applied mechanical models at the scale of Brownian motion. In fact, given that they utilize thermal energy to produce work from these

[48] Further, from Huxley's perspective, the formal aspects of these analyses are meant only to facilitate vetting of the mechanical models and to aid in formulating further such models. As we discuss further in our conclusion, we take this perspective to be a philosophically valuable one.

mechanisms, it *must* be the case that thermal energy and mechanical movement are at the same scale so that mechanical parts move due to Brownian motion. In other words, contrary to what Nicholson suggests, not only *can* we understand these as mechanisms *in spite of* their being subject to thermal forces, we *must* understand them at that scale in order to understand how the mechanisms operate in the first place.

Shortly after Vale and Oosawa's publication, new data indicating the structure of myosin in different nucleotide states was interpreted in light of Hugh Huxley's swinging crossbridge model. This data was provided not by electron microscopy, which to that point had provided much of the information about the structure of molecular motors, but protein crystallography. Crystalizing myosin in a state they interpreted as representing its rigor state in which it was still attached to actin but had expelled ADP and Pi, Rayment, Rypniewski, et al. (1993) generated an image that showed the ATP binding site (labeled Nucleotidebinding site in Figure 5) at the opposite end of a β-sheet from the Actinbinding cleft, situated at the end of a cleft between the Lower 50 K and the Upper 50 K domains. The images also revealed a long helical tail, consisting of an α helix having the appearance of a lever arm, rigidly attached to the converter domain.

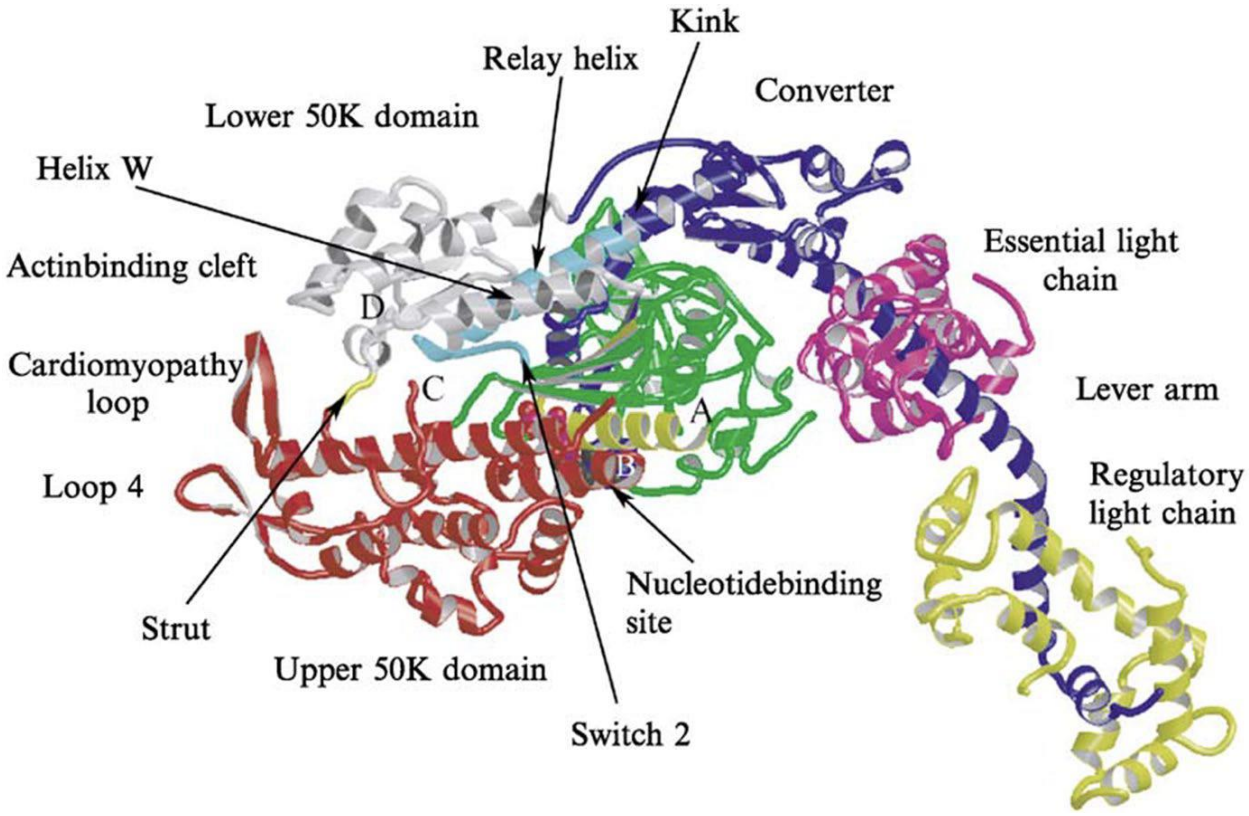

Figure 5.5: Ribbon diagram of myosin motor domain in post-rigor state based on Rayment, Rypniewski, et al. (1993). Reprinted from Advances in Protein Chemistry, Vol. 71, Geeves, M. A. & Holmes, K. C., The molecular mechanism of muscle contraction, Fig. 2, Copyright (2005), with permission from Elsevier.

Drawing on this image of the atomic structure of myosin, an image of the atomic structure of actin by Kabsch, Mannherz, Suck, Pai, and Holmes (1990), and earlier EM studies, Rayment, Holden, et al. (1993) proposed a model that involved the transfer of force between the ATP binding site and the actin binding site through the β-sheet between them and between them and the lever arm. As seen in Figure 6, they proposed that the conformation of myosin changes with the hydrolysis of ATP, moving the lever arm to the raised position (panel B to C). They further proposed that the lever arm changes again with the expulsion of Pi, returning to its original position (panel C to D). This model was referred to as a "swinging lever arm model." Subsequently, researchers were able to obtain images of myosin when bound to ATP (Smith &

Rayment, 1995) and after ATP hydrolysis (Smith & Rayment, 1996), conditions assumed to precede the powerstroke. By comparing these additional images with the first image, researchers were able to see that the converter and lever arm had shifted by 60–70°. This angular difference was proposed to correspond to the 10 nm movement myosin was observed to impose on actin (Holmes, 1996, 1997) and so to vindicate the occurrence of a powerstroke.

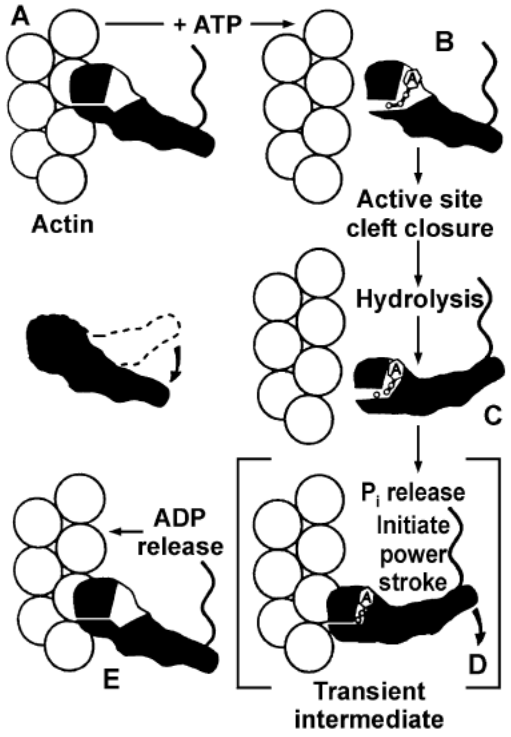

Figure 5.6: Swinging lever arm model of how hydrolysis of ATP in myosin generates a power stroke. From Rayment, Holden, et al. (1993)

During the 1990s much of Vale's focus was directed not at myosin but at another molecular motor that he had identified as responsible for the transport of cargo along microtubules in axons—kinesin (Vale et al., 1985). By the end of the 1980s he and his colleagues had developed evidence that the two monomers of kinesin moved sequentially along the microtubule in what they characterized as a hand-over-hand fashion (Howard, Hudspeth, & Vale, 1989). During the same period as Rayment and colleagues were developing images of the

molecular structure of myosin, Vale and his colleagues produced images of the molecular structure of kinesin that revealed that its motor domain is very similar to that of myosin (Kull, Sablin, Lau, Fletterick, & Vale, 1996). The researchers appealed to this similarity to identify a candidate microtubule binding site, suggesting that a conformation change induced during ATP hydrolysis also exerts force that changes the ability of kinesin to bind a microtubule. Explaining the locomotion of kinesin was more challenging. Kinesin does not exert force on the microtubule in the manner myosin does on actin; rather, force is directed at moving one kinesin monomer ahead of the other. The responsible force is thought to be generated in the linker connecting the two monomers. Two images of the crystal structure of the linker, one from rat (Kozielski et al., 1997) and the other from human (Kull et al., 1996) suggested that the linker could change conformation. At the time it was not possible to crystalize the linker while connected to the head in different stages of ATP hydrolysis, so Vale and his collaborators used spectral analysis to show different conformations of the neck linker when kinesins are in different nucleotide and microtubule binding states (Rice et al., 1999). These researchers concluded that the neck linker is generally in an unstructured and so flexible state but becomes more ordered and immobile when the microtubule bound kinesin binds ATP. As a result of being in this structured state, they inferred that one kinesin head exercises a force on the other head, moving it forward.

In this work on kinesin, Vale has clearly embraced the key idea of the powerstroke model: that ATP hydrolysis produces movement by changing the molecular conformation of the linker. This is not inconsistent, however, with acknowledging a role for thermal energy. In fact, in their discussion they refer to ATP hydrolysis as "providing the energy source for rectifying this Brownian ratchet" (Rice et al., 1999, p. 783). To reconcile this reference to a Brownian ratchet with his invocation of a powerstroke, one needs to recognize that an important aspect of kinesin's

movement is that the unbound head fluctuates randomly due to thermal forces. ATP binding to the bound head induces the neck-linker conformation which biases the motion of the free head in the direction of the next binding site on the microtubule. Once the free head finds the binding site due to Brownian motion, it binds to the site, releases ADP, and is capable of binding ATP for the next cycle. Thus, rather than understanding ATP energy as serving to introduce an apparent "temperature difference" as Vale had suggested in his ratchet account of myosin, in his account of kinesin, ATP energy is used to induce a conformational change in the linker—a powerstroke—which generates force to situate the stepping head close enough to the next binding site that Brownian motion will find it. In treating this process as analogous to a Brownian ratchet, therefore, Vale and his colleagues do not claim that the movement of the kinesin head is due solely to Brownian motion. Rather, they continue in the next sentence to assert that "force generation in the monomer occurs upon ATP binding" indicating that the nucleotide binding of the forward head generates force that gives the rear head's motion its direction. Vale's commitment at this point to the powerstroke model is even clearer in a paper to following year:

> Just as in an automobile, the site that processes the chemical fuel must be linked through intermediate components to the site that ultimately generates the motion. In the automobile, the breakdown of the chemical fuel is coupled to the stroking of a piston, which in turn is linked through the crankshaft and transmission to the turning of the wheels. A somewhat analogous situation for translating chemical changes into mechanical motions exists in molecular motors (Vale & Milligan, 2000).

In this section we have shown that Andrew Huxley and Vale and Oosawa offered Brownian ratchet models of myosin's movement along actin as hypothetical models. They were, first of all, treated as mechanical models. While they were not defended as literal models, they were used to develop mathematical analyses. Neither group of researchers set them opposed to

powerstroke models. Once protein crystallography revealed structures within myosin (lever arm) and kinesin (neck linker), Vale embraced the interpretation that the motors operated through a powerstroke. Yet, even then his powerstroke account was incorporated into an account that acknowledged a role for thermal energy.

Section 3: Brownian Ratchet Models as Alternatives to Powerstroke Models

In this section we take up a subsequent generation of Brownian ratchet models advanced as competitors to powerstroke models. These models are motivated in part by conceptual and empirical challenges to powerstroke models. Nicholson identifies three such challenges, none which is decisive. First, he emphasizes the difficulty of moving in the face of Brownian motion, quoting Astumian's claims that it is like swimming in molasses or walking in a hurricane. The alternatives he presents are either for motors to work in opposition to Brownian motion or to make use of it. Conceptually, he argues that the free energy made available by ATP hydrolysis is only an order of magnitude above Brownian motion buffeting them. However, he doesn't explain why this is not sufficient to generate the motor's movement. As we saw in Vale's powerstroke model of kinesin, ATP energy need not work *in opposition* to thermal forces. On Vale's model, the role of ATP energy is analogous to the role it plays on Vale and Oosawa's ratchet model. On the latter, it induces an "apparent temperature difference" across actin and myosin filaments which is, in turn, analogous to the temperature differences across the containers on Feynman's ratchet model. ATP energy, then, has a task to perform no more arduous than that of the free energy required to maintain a temperature difference in Feynman's ratchet. The ATP energy doesn't need to be sufficient to power "walking in a `hurricane." It need only provide sufficient energy to advantage otherwise random Brownian movement in a particular direction. Second, he

appeals to evidence (Pierce, Hom-Booher, Otsuka, & Vale, 1999) that monomeric kinesins, in which there is no linker that could undergo conformation change, can move processively. The evidence has been challenged (Schimert, Budaitis, Reinemann, Lang, & Verhey, 2019; Xie, 2010; Huang, Vega, & Gopinathan, 2011; Hammond et al., 2009). Finally, he objects that the hydrolysis of a single ATP is associated with different sized steps and that the structural geometry of myosin does not correspond to its step size. The adjudication of these claims is not straightforward, but for Nicholson they suffice to motivate taking the Brownian motion account as a correct and non-mechanistic alternative to powerstroke.[49]

Instead of debating these claims against powerstroke models further, we focus on the alternative Brownian motion models, beginning with Nicholson's characterization of these models and then considering those advanced by theorists to whom he appeals. As his exemplar of non-mechanical Brownian motion models, Nicholson invokes what are known as flashing ratchet models, first advanced by Ajdari and Prost (1992). These models propose that what hydrolysis of ATP does is switch the motor between two energy landscapes. As shown in the middle of Figure 7, one landscape is flat and when the motor is in that landscape, the molecule is free to diffuse equally in all directions as a result of Brownian motion. The Gaussian curve shows the probabilities of it diffusing to each location. The other landscape exhibits an asymmetric

[49] Nicholson does not mention a variety of findings that present challenges for Brownian ratchet accounts. Hwang and Karplus (2019) review several of these with respect to kinesin. Perhaps the most serious is theoretical: while the kinesin head can move the necessary 16 nm to the next microtubule binding site with diffusion, it is unable to do so when there is a load (i.e., when it is transporting cargo). The time it would take to reach 16 nm increases exponentially with load. Moreover, in experiments with fluorescently tagged motor heads, Mori, Vale, and Tomishige (2007) observed that the rear head stays behind the other. Noting several other experiments with similar results, Hwang and Karplus conclude "Brownian motion is not sufficient to push the head forward. The moving head spends most of the time located on the right side of the MT-bound head relative to the walking direction." They offer a mechanistic explanation "In the nucleotide-free MT-bound head, the base of its neck linker is located behind the α4 helix, which prevents forward motion. Binding of an ATP causes the head to tilt leftward, so that the base of the neck linker is lifted over α4, allowing access to the forward-pointing state." Additional evidence involves the torque generated in kinesin movement. It is hard to see how Brownian diffusion would create torque. It rather points to a powerstroke.

sawtooth structure, with the lowest point representing an attractor in the dynamical systems sense. Whatever attractor the motor is in when this landscape is turned on, it is most likely to move to the bottom of that attractor, resulting in a much narrower Gaussian curve of positions to which the motor can move as a result of Brownian motion. The cycling between these two landscapes is able to generate directional movement: When the attractor landscape is flattened, the motor sometimes moves to the right so that when the sawtooth landscape is reinstituted, the motor is trapped in a new attractor and will move to its minimum. Although it will not move right on each cycle, it will seldom move in the reverse direction and over many cycles will move right.

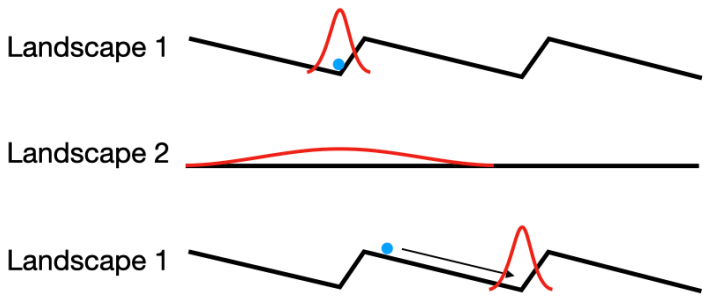

Figure 5.7: Flashing ratchet model. When in landscape 1, the probability that the particle (blue) will move to the next attractor by Brownian motion (red Gausian curve) is very small. In Landscape 2, when it moves by diffusion, that probability is much greater. If it makes it to the next attractor before Landscape 1 is reinstituted, it will follow the downward trajectory. When it reaches that attractor, again its probability of moving to the subsequent attractor (or returning to the previous attractor) is low.

Other than telling us that ATP hydrolysis is what generates the switching between the two landscapes, Nicholson doesn't explain what the landscapes consist in. It is clear, though, that he does not view this switching to require mechanistic detail:

> In this model, 'structure' and 'specificity' do not play the same critical role in determining how the protein moves as they do in the . . . power-stroke model. . . As there is no specific reference to the topological or geometrical configuration of the motor protein (other than to its alternative energy profiles, which do have a structural basis),

> there is no need to speculate about how its various structural domains interact with one another in a perfectly synchronized fashion to generate motion. Similarly, because there is no crucial mechanical step—no power-stroke—that can be identified as the specific moment at which chemical energy is transformed into work (2019, p. 118).

To assess whether Brownian ratchet accounts do, indeed, live up to these strong claims, we will turn to two of the scientists whom Nicholson draws upon: Astumian in this section, Ait-Haddou and Herzog in the following section.

Nicholson references Astumian (2001), a paper in *Scientific American*. We begin with that and then turn to some of Astumian's more technical discussions. One of the first things to note is that the focus of Astumian's discussion is not Brownian motion itself but how to take advantage of random movement to achieve directional movement. Despite his strong claims about the different forces at work at the macroscale, Astumian illustrates the process of deriving directional motion from random movement with a macroscopic example involving moving a car uphill by taking advantage of hail hitting the car and nudging it in different directions. He envisages a driver putting a brick behind the tires every time it randomly moves forward. He does not analyze the work required of the driver in recognizing movement in the right direction and inserting the brick. Instead, he offers a further example that does not require a cognitive act of recognition but simply a driver repeatedly pumping a brake. When it is off, the car moves backwards or forwards depending on what hail hits it. But when the driver applies the brake, a piston is forced into a gear with teeth skewed as in the flashing ratchet, with a gradual slope in the backwards direction and a steep slope in the forward direction so that the lowest point is closer to the front tooth. The jamming of the brake into the gear serves to move it to this lowest position near the front tooth. When the brake is briefly released, hail will sometimes move the car upwards sufficiently so that when the brake is reengaged, it will be beyond the next tooth. But given the longer distance to the previous tooth, it is unlikely to roll backwards. In this

characterization of the flashing ratchet, it becomes clear that a major factor in the car moving up the hill is the force applied to the brake: as a result of the skewing of the teeth, it is this force that pushes the car down to the minimum, thereby moving the car up the hill. This force derives from a source of free-energy—the person pumping the break. This force plays a role in Astumian's account analogous to that of the actual temperature differences between boxes in Feynman's ratchet, as well as to the ATP-induced apparent temperature difference between actin and myosin filaments in Vale and Oosawa's ratchet model, and finally to the ATP-induced powerstroke that occurs in the kinesin neck-linker on Vale's later model.

In fact, Astumian (2016) makes precisely this point in deploying the same car example. In the caption to the figure illustrating the example he states the following with respect to what he there calls the "energy ratchet": "Note that the energy comes not from the hail itself, but from the effort expended by the driver in applying the brake—that is, from a power stroke" (p. 1727). Thus, like the demon in Maxwell's original thought experiment, a source of free energy, not merely Brownian motion, is needed to drive the car forward. When Astumian (2007) invokes the energy ratchet to explain kinesin movement, he acknowledges the role of the sawtooth shape: "the sawtooth shape . . . can push the molecule forward." Of course, the shape does nothing itself, but only constrains the energy from the driver depressing the brake.

Astumian (2016) introduces yet another variant ratchet model which he calls the "information ratchet." In this version, applying the brake does not generate movement, but restricts when the car moves. If the driver differentiates when the car is at the upper end of the range and briefly releases the brake at that moment there is a chance it will move to the next cog and little chance it will roll back a whole cog. Again, Astumian does not elaborate on the energy involved in such an information ratchet or by what manner of information processing it might

operate. For Astumian, however, that is not an issue. He is not concerned to argue against mechanisms, even ones using free energy to apply force, in explaining the overall activity of molecular motors. Rather, he sharply distinguishes locomotion and the stopping of that movement. While he argues that Brownian motion suffices for the movement of the motor, he acknowledges that it cannot explain its stopping at the target location. He explicitly says: "energy is used to cause a cessation of motion" (Astumian, 2007, p. 57) and refers to the "ratchet mechanism" (p. 59). To apply this to molecular motors, Astumian (2000, p. 1720) invokes Brownian motion of atoms within the motor molecule so that the changing conformation that is observed might be due solely to Brownian motion. ATP hydrolysis acts like the brake, stopping the molecule in the conformation corresponding to the motor's motion.

Despite his contention that one cannot carry out the same style of mechanical analysis at the nanoscale as one does at the macroscale, in his numerous publications Astumian attempts to provide an intuitive understanding of how Brownian motion produces movement through space using a macroscale example of moving a car relying on random perturbations by hail. Astumian might respond that these are merely meant to generate intuitions and that what really matters is his mathematical analysis of the relevant physics. Astumian, Mukherjee, and Warshel (2016) equate the physics of a molecular motor with its equation of motion ("the "physics" of a chemically driven molecular machine—its equation of motion"):

$$\frac{d\vec{r}}{dt} = -\gamma^{-1}\nabla U(\vec{r}) + \sqrt{2D}\,\vec{f}(t)$$

In which $\vec{r}$ is a vector representing the relevant degrees of freedom, γ is a coefficient specifying the viscous friction, $-\nabla U(\vec{r})$ is the force due to the energy surface $U(\vec{r})$, and $\vec{f}(t)$ corresponds to random thermal noise. Starting from this equation, the authors develop what they claim is an

account of molecular motors. They assert: “All of the information about how the structure relates to the mechanism is contained in the energy function U(r).” Based on this analysis, they argue that the powerstroke is irrelevant to the analysis of chemically driven motors (see also Astumian, 2015).

We contend that Astumian’s car examples have the same status as Huxley attributes to his speculative mechanical models. For Huxley, although he acknowledges their speculative nature, his models nonetheless represent substantive hypotheses about how the mechanism might work that will likely prove incorrect in light of further empirical investigation. Subsequently, new models would be proposed and, ideally, researchers will settle on a consensus mechanistic explanation. From this point of view, Astumian’s philosophical gambit is to put forward just such a speculative mechanical account but label it as a mere metaphor, relieving it of the epistemic duties Huxley would assign it—denying that it has any other epistemological role to play beyond that of “making intuitive” the mathematics. Astumian’s gambit is thus to insist on the explanatorily privileged status of his equations and claim that, therefore, the messy details of its mechanical implementation are explanatorily irrelevant, though we might allude to them when it helps to make the math intuitive.

We take cues from Huxley in taking this to represent a misunderstanding of the status of speculative mechanical models vis-à-vis their formal analysis. Following Huxley, we take the models, though speculative, to represent substantive mechanical hypotheses to be vetted in the light of further mechanistic experimentation. Also following Huxley, we take the formal analyses of these speculative models to lend plausibility to the mechanisms in showing that the mechanism, though speculative in its mechanistic details, is conceptually consistent with our understanding of the same system at a different scale. From this Huxley-inspired point of view, a

view like Astumian's pre-maturely forecloses the possibility that insight can be achieved by the dual formal-and-mechanical analysis characteristic of Andrew Huxley's approach.

The foundation for Astumian's mathematical analysis is the assumption that molecular motors are "mechanically equilibrated systems" which exhibit microscopic reversibility: at equilibrium every process is as likely as the exact (microscopic) reverse of that process. The equation he advances and the further mathematical model of motor motion is based on the assumption that the motion of molecular motors is reversible. This principle has been challenged. As developed by Hwang and Karplus (2019), reversibility is not a universal principle, but a physical property that applies only to some systems. In particular, reversibility applies only to systems at equilibrium, but motors are not necessarily at equilibrium: "conformational states integrate atomic degrees of freedom, for which there is no guarantee that microscopic reversibility holds. Which transitions during the motility cycle can be considered reversible or irreversible depends on the specific motor protein in question."

Baker (2022a) provides an illuminating perspective on Astumian's approach. Baker contrasts powerstroke accounts and Astumian's Brownian ratchet model as a basis for advancing a third alternative. For him, reversibility is not a foundational principle, but a phenomenon observed empirically: when forces are applied in opposition to the movement of the motor, ATP hydrolysis can be reversed and ATP synthesized (this is illustrated in the FOF1 ATPase that, when driven backwards by protons crossing the mitochondrial membrane, becomes an ATP synthase). He argues that this has made powerstroke models of myosin problematic since they hypothesize that an irreversible process occurs within the motor. Potential energy is stored in a spring-like structure that extends between the lever arm and the rest of myosin. When Pi escapes, that energy is released in an irreversible powerstroke. Once released, force applied to the lever

arm cannot be used to re-synthesize ATP. In Panel A of Figure 8 Baker represents the reversible buildup of the spring's energy as a result of $\Delta G_i(w_{rev})$ acting to close the cleft in the actin binding site. Pi prevents the spring from relaxing. When Pi is subsequently released, the spring compresses, corresponding to the powerstroke, and exerts force on actin to move it a distance x against the fixed force F on actin. The relaxing of the spring represents the irreversible step. In contrast, the Brownian ratchet account, which Baker refers to as Chemical Fx (Panel C), maintains reversibility throughout, even after actin is moved. there is no irreversible step in the process. Notably, Panel C does not include any representation of changes in how the lever arm relates to the rest of myosin. That reflects Astumian's claim that any powerstroke is irrelevant to the movement of the motor.

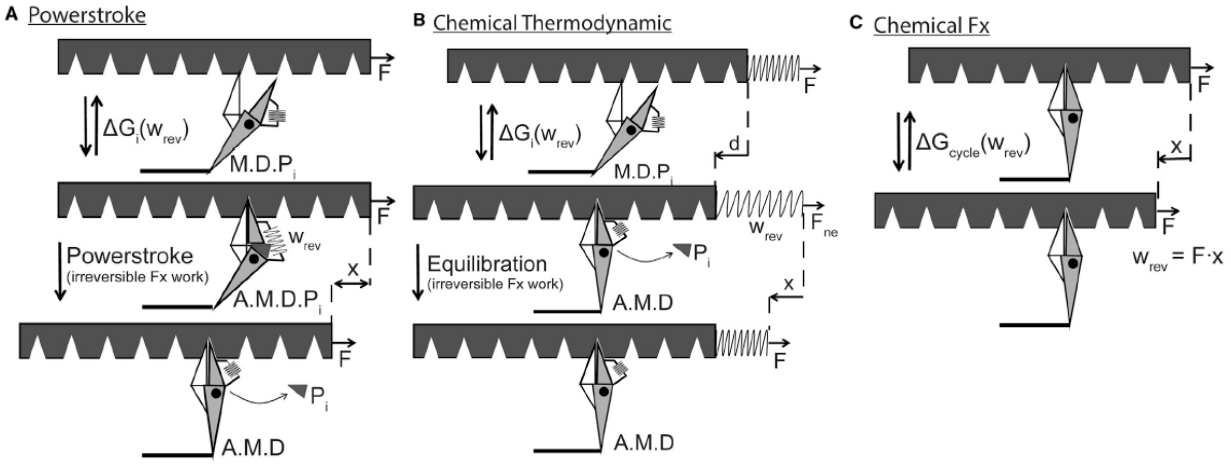

Figure 5.8: Baker's comparisons between models

Baker represents his alternative, which he calls a chemical thermodynamic account, in Panel B. Again, no powerstroke within the motor is represented. Even through Pi is shown escaping from the motor after it binds actin, it is not shown as impeding the movement of myosin before it is released. The closing of the cleft and binding of myosin to actin is presented as

moving actin, performing reversible work that generates tension in a spring stretched out between actin and a moveable anchor outside the actomyosin structure. The force F remains constant because, after every one of myosin's force generating steps, the spring first stretches and then the moveable anchor is pulled along to catch up like an inchworm. This "catching up" represents the tension in the spring relaxing. On Baker's model, this relaxation is the irreversible work performed by the system.[50]

Both Astumian and Baker frame their alternatives to the powerstroke model by assuming that the behavior of myosin in response to ATP hydrolysis is reversible. The apparent disagreement between them is that Baker argues that irreversibility enters in the larger system whereas Astumian's formal analysis does not introduce irreversibility at all. But Astumian does invoke ATP hydrolysis to stop the movement of the motor, although this is only spelled out mechanically at the level of his car metaphor and then promptly formulated as the abstract, formal, "energy function, U(r)." Whether introduced by way of metaphor or not, however, it is precisely ATPs contribution of free-energy that renders the movement of the motor irreversible. So, though it might not be explicitly represented in his equations, irreversibility remains in the background against which his equations are intelligible as not mere empty formalisms, but as descriptions the actual systems they are intended to describe. In other words, irreversibility is indispensable to the intelligibility of Astumian's formal "explanation," but he smuggles it in at precisely the level of mechanical implementation details that his own philosophical approach to understanding motors deems irrelevant.

[50] Although we do not pursue them, issues of holism vs. mechanism are at stake here: "thermodynamic forces are contained within the walls that constrain them (within system springs) not within molecules that somehow hold their own force (within molecular springs), demonstrating that corpuscular mechanics attributes to molecules the mechanical properties of the system that contains them" (Baker, 2022b, p. 15906)

With this in mind, the difference between the powerstroke model, Baker's model and Astumian's reduces to where irreversibility is localized in their models—within myosin itself, within the larger environment (e.g. Baker's external spring) or wherever it is localized in Astumian's metaphors, namely, in the application of force to a gear tooth. For our purposes, it suffices to note that on all accounts the introduction of an irreversible step invokes a mechanism—the constrained application of free energy. But we should note a further point—the key claim of the powerstroke account is not the production of motion but the use of the energy from ATP hydrolysis to generate *directional* motion. In this sense, all agree that, as Maxwell taught, energy from ATP hydrolysis is required for molecular motor systems to function—Brownian motion alone does not suffice.

We return to this issue in the next section, but first consider a further criticism Baker (2022b) offers of powerstroke models. He invoke Gibbs' (1902) expression "rational mechanics" to indicate why powerstroke accounts are misguided. Gibbs used this phrase to characterize the misguided attempt to attribute phenomena such as changes in energetic states in a system to the behavior of individual particles within that system.[51] Gibbs argument against rational mechanics focused on the massive number of participles involved in phenomena such as temperature in a gas. Baker quotes Gibbs as presenting the laws of thermodynamics as required for "beings who have not the fineness of perception to enable them to appreciate quantities of the order of magnitude of those which relate to single particles." Baker argues that same conclusion applies to molecular motors "[b]ecause we are incapable of easily comprehending the energetic changes

[51] He breaks with Astumian, though, in allowing the application of mechanical ideas such as springs to the whole system and in viewing the enzymatic activity of the motor as indeed relevant to the forces generated. We will not further develop these differences here. An interesting objection Baker raises against Astumian is that he illicitly substitutes the number of motors for the number of ATP molecules in the expression characterizing the force that will cause motors to stall: $F_0=-N_{ATP}\Delta G_{ATP}+Fx$

associated with the distortion of every atomic bond that occurs within a protein."[52] This objection would be relevant if a powerstroke was characterized in terms of every atomic bond within a protein, but the proposal for powerstrokes are motivated by identifying, through protein crystallography, conformational changes at the scale of tertiary or quaternary structure of protein. At this scale, the physical changes generated by ATP hydrolysis and again with the liberation of Pi are identifiable and describable. The justification for mechanical analysis in this case is that one can identify the major changes that occur and characterize these changes in mechanical terms like "powerstroke."

In this section we focused on how Nicholson, Astumian, and Baker all propose to explain the operation of molecular motors such as myosin while rejecting powerstroke models. For Nicholson, the objective is to show that the activity can be explained without appeal to mechanisms. This was not the focus for Astumian, who is clear that the activity of stopping a molecular motor at the appropriate target requires a mechanism that employs the free energy released by ATP hydrolysis. His objective is to explain the *locomotion itself* solely in terms of Brownian motion. His account assumes microscopic reversibility and his equations respect that principle. But the overall movement is irreversible, and he smuggles irreversibility in at a level that his, but not Huxley's, philosophical approach to molecular motors deems explanatorily irrelevant. However, although his philosophical approach involves this tension, it nonetheless

[52] Astumian et al. (2016) advance a similar argument: "Many authors, however, seem to be looking for a description in terms of classical mechanics, and this is what cannot be given, for the simple reason that the problem of mechano-chemical coupling by an enzyme is NOT a problem of classical mechanics. It makes almost as little sense to seek a mechanical description of the coupling between a chemical reaction and the motion of a molecular machine in water as it does to seek a mechanical description of the diffraction of an electron. . . In a full molecular dynamics simulation involving all degrees of freedom of both the protein and of the molecules in the solution, the dynamics would be described by Newton's equations of motion in which acceleration and not velocity appear, but the impracticality of a classical mechanical description in terms of Newton's equations (or Lagrange's or Hamilton's) is overwhelming. There are 10^{18}–10^{20} collisions each second between water molecules and a molecular machine like myosin, the flagellar motor, or kinesin, and any attempt to model the system in terms of Newton's equations for longer than a few picoseconds is doomed to failure."

includes some irreversible step—that his metaphors do not precisely localize—related in some way to the contribution of free energy. And this is just a powerstroke. We discussed Baker's analysis, since it helpfully focuses on where the action of the motor becomes irreversible as one question about where mechanism enters. Moreover, his model, like the powerstroke models, appeals to movement generated by ATP hydrolysis to explain locomotion, and we suggested that this feature, rather than the introduction of an irreversible step, may be seen as the key contention of the powerstroke account (that is, Baker's model can be viewed as a variation on the powerstroke framework).

Section 4: Locating Powerstrokes within Brownian Ratchet Models

We turn in this section to a second example of Brownian ratchet accounts to which Nicholson appeals. Nicholson attributes to Ait-Haddou and Herzog (2003) the claim "motor proteins are able to move directionally in the absence of mechanical forces" (Nicholson, 2020, p. 57). This seriously misrepresents their position. Ait-Haddou and Herzog do frame their analysis in terms of Brownian motion and argue that given the size of molecular motors, this is an important factor in explaining their behavior. Their model is developed to include Brownian motion. But, contrary to Nicholson's portrayal, they then provide a role for a powerstroke within their Brownian motion account.

A key element in Ait-Haddou and Herzog's analysis is a rejection of deterministic models of the operation of molecular motors—they maintain that because "the many small parts that make up molecular motors, including the weak bonding in its tertiary and quaternary structure, must operate at energies only marginally higher than that of the thermal bath, and hence are subjected to large fluctuations" (2003, p. 193), a probabilistic analysis is required. They advance

such an analysis of the movement of motors that, like that put forward by Astumian (2007), draws on the flashing ratchet framework discussed above and shown in Figure 7.

Unlike Nicholson and Astumian, Ait-Haddou and Herzog explicitly develop how two critical features of the flashing ratchet models are instantiated in the physical structure of myosin—the process of switching between energy landscapes and the activity during the ON state in which the motor moves to the bottom. (All Astumian and Nicholson offer is the claim that ATP hydrolysis is responsible for the switching between the ON and OFF states). In doing so, Ait-Haddou and Hertzog draw upon the mechanistic accounts of myosin developed on the basis of Rayment et al.'s x-ray crystallography studies. They embrace the hypothesis that the binding of ATP to myosin results in a conformation change that enables myosin to bind actin and that the subsequent hydrolysis of ATP results in release from actin. To explain the downward slope in the ON state (when myosin is bound to actin), they appeal to the movement of the lever arm hypothesized by Rayment et al. In both their 2002 and 2003 papers they devote a paragraph to describing the powerstroke and reprint Figure 6 above from Rayment, Holden, et al. (1993), in which the powerstroke is clearly labeled. In the text of their 2003 paper they state "Following the release of phosphate, the myosin head is thought to pull the actin filament past the myosin filament. During this process, the myosin head undergoes conformational changes, referred to as the power stroke. Following the power stroke, ADP (adenosine diphosphate) is released, then ATP attaches again to the myosin head, and the contraction cycle starts again."

After connecting the flashing ratchet account with powerstroke models, Ait-Haddou and Herzog put forward a two-state model. Here we abstract from the equations and focus on Figure

9, which they adapt from Jülicher (1999)[53] and label "Ratchet model for muscle contraction."

They narrate the processes identified in their caption to the figure:

> In the attached state, the myosin head is located in a local minimum of the potential of interaction between actin and myosin filament (potential VA(x)) (1). Once the ATP attaches to the myosin head, the myosin head detaches from the actin filament and undergoes a free Brownian motion (2 and 3). After ATP hydrolysis, and because of the asymmetry of the potential once the myosin head is attached to the actin filament, the myosin head is mostly found in a region of negative slope on the potential VA(x), allowing the fiber to shorten and produce force (4).

A significant feature of this two-state model is that Brownian motion only figures in one of the two states while the powerstroke is operative in the other state.

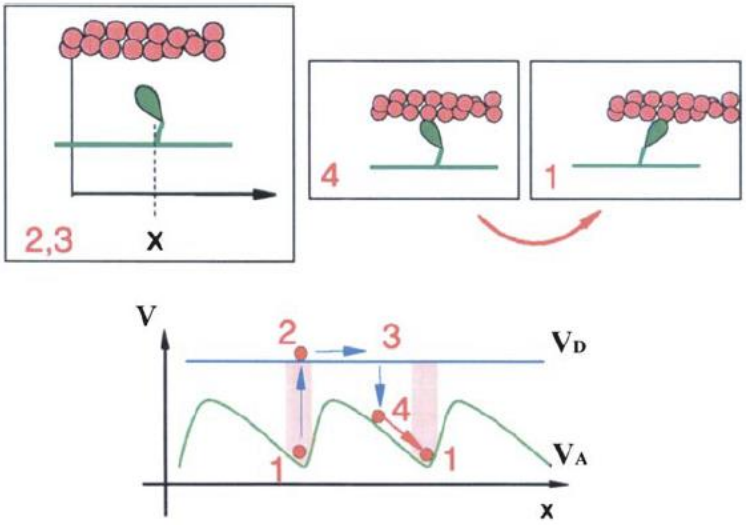

Figure 5.9: Ait-Haddou and Herzog's (2002) figure *Ratchet model for muscle contraction*, which they reprint from Jülicher (1999) with the minor change of relabeling W_1 and W_2 as V_A and V_D. See text for Ait-Haddou and Herzog's figure caption.

In advancing a framework that makes Brownian motion central, Ait-Haddou and Herzog's presentation may be different from that of other proponents of the powerstroke account. Yet, as nearly everyone agrees that Brownian motion is a factor in motor movement, an

[53] Julicher does not discuss Brownian motion or thermal noise, but simply presents himself as advancing a stochastic model of "force and motion generation of molecular motors." Julicher clearly embraces the powerstroke model, referring to a "force generating step (power-stroke)."

integrated account is not alien to the powerstroke tradition. Ait-Haddou and Herzog do depart from those who treat Brownian motion as serving largely to enable myosin to locate the binding site on actin, treating it as instead as positioning the motor in a location in which the powerstroke can produce forward movement. For our purposes, however, this difference does not matter. What is clear is that, far from rejecting the powerstroke model, Ait-Haddou and Herzog embrace it and incorporate it within the larger framework which also allows for Brownian motion.

Brownian motion of course does not cease when myosin binds to actin. Ait-Haddou and Herzog, though, provide a reason why it can be ignored in characterizing the powerstroke, a reason that further answers Nicholson's, Astumian's, and Baker's contention that macroscale concepts such as mechanical force generation are not appropriately applied to the nanoscale. They acknowledge that the weak bonds (hydrogen, van der Waals, ionic etc.) that determine the tertiary and quaternary structure of a protein have a strength only marginally greater than the energy of the thermal bath and that, therefore, the protein will be in a continual state of fluctuation. They further acknowledge the contention that led Baker to invoke Gibbs' derogatory characterization of powerstroke accounts as engaged in "rational mechanics": "a detailed description of all conformational variables [atomic positions, bond angles, bond distances, etc.] presents an unrealistic computational challenge." Ait-Haddou and Herzog argue, however, that in their model, the variables relevant for characterizing the conformational changes of the protein are "characterized by great relaxation times to the equilibrium compared to the other, neglected [atomic level] conformational variables." From this they infer: "Conformational variables that have quick relaxation times can be ignored because they are considered to be in equilibrium within the time scale of the relaxation of the heat bath fluctuations" (p. 201).

Interestingly, in advancing their process framework, Dupre and Nicholson assert that while ". . . *everything flows* . . . this is not to say that everything flows *at the same rate.*" What Ait-Haddou and Herzog's analysis shows is that rate matters in assessing which processes are relevant to which phenomenon. The relatively slow conformational changes induced by ATP hydrolysis don't negate the faster Brownian movement. But given the lack of directionality of Brownian motion, the fast timescale of thermal fluctuations means that those different movements will cancel out at the timescale of conformational change of the molecule. Accordingly, they can be treated as at equilibrium at that timescale and dominated by the conformational changes induced by ATP hydrolysis. Thus, the mechanical idea of a "powerstroke" is consistent with the processualist's view that we can individuate processes according to their characteristic rates. The mechanist view insists, however, that the "slower" processes are appropriately treated as mechanistic components for the reason that they involve constraints on the flow of free energy. In other words, for the mechanist, the difference is not just a matter of rates, but a matter of mechanical function in the system which, in turn, is a matter of how the mechanism is organized to as to enable its metastable parts to perform these different functions.

Far from rejecting a powerstroke analysis and a mechanistic account of the movement of molecular motors, we have shown in this section that Ait-Haddou and Herzog provide a framework in which a powerstroke is integrated into a model that also recognizes the role of Brownian motion. Instead of just abstractly referencing a flashing ratchet model, they explain both the switching and the movement to the attractor in the ON state in terms of structural features of myosin that constrain its ability to bind actin and to move against it. They further note the importance of attending to the timescale—at the slower timescale of the conformation change

induced by ATP hydrolysis, the faster timescale Brownian motion within molecules at the level of primary structure happens in different directions but cancel out and can be left out of the analysis.

Section 5: What Do Molecular Motors Teach us about Processual versus Mechanistic Explanations?

Processual theorists advance their accounts as a preferred alternative to mechanistic explanations in biology. Nicholson has used molecular motors to illustrate what a processual account would be: instead of treating the motors as moving through a mechanical powerstroke, they would be understood as utilizing Brownian motion. He contends that accounts that draw on Brownian motion provide non-mechanical accounts. To assess this claim, we began by examining how the idea of a Brownian ratchet figured in the work of two pioneers in molecular motor research, Andrew Huxley and Vale. Both of these investigators advanced mechanical analyses of how Brownian motion could figure in explaining the operation of molecular motors that were intended to guide their mathematical theorizing. While they represented serious empirical hypotheses, the researchers who put them forward understood them to be speculative and beyond the reach of empirical investigation at the time. That they were treated as serious empirical hypotheses is reflected in the fact that both theorists dropped them when later empirical techniques enabled suitable powerstroke accounts to be developed. We then turned to the proposals for Brownian motion accounts advanced after detailed powerstroke models were available. We reviewed not only Nicholson's own appeal to flashing ratchet models, but the models proposed by the scientists on whom he draws. Astumian's primary goal is to appeal to Brownian motion to explain locomotion, for which he offers a detailed mathematical account

based on the assumption that the motor exhibits microscopic reversibility. Though Astumian denies it any explanatory significance he is clear that a mechanism powered by ATP is required to rectify Brownian motion. We illustrated the tension in such an account by comparing the approach it reflects with Andrew Huxley's and endorsed the latter's dual mathematical-mechanical methodology. We introduced Baker's more recent analysis, which helpfully focuses attention on how reversible processes become irreversible and shows that, even if one thinks the locomotive activity is reversible, it can be powered by energy from ATP hydrolysis. Finally, we examined the contributions of Ait-Haddou and Herzog, who explicitly build a powerstroke into their analysis that is overall framed in terms of Brownian motion. Our conclusion is that, far from providing a non-mechanical account of the activity of molecular motors, even those appealing to Brownian motion appeal in crucial ways to mechanistic ideas.

We conclude by considering the question of how to conceptualize the relation of processual and mechanistic explanation. A key concept appealed to in processual accounts is flow. Nicholson and Dupré (2018) entitle their book *Everything Flows*. Flow, on their account, involves change and they invoke Heraclitus as providing an apt account of nature. A key question to ask is what is responsible for flow. The most plausible candidate in contemporary physical science is energy. Brownian motion, to which they appeal, represents thermal energy. The challenge is to get directional flow—flow that can do work—out of thermal energy. As we have seen, even accounts of molecular motors that draw on Brownian motion for locomotion of the motors recognize that to get directionality out of thermal energy, work is required, which requires a different form of energy, Gibbs free energy, provided by ATP.

Gibbs free energy on its own flows—it dissipates. This is what is captured in the laws of thermodynamics, especially the second law that specifies that in an open system, entropy

increases. To use Gibbs free energy to perform work, its flow must be constrained. Winning and Bechtel (2018) characterized mechanisms as systems that constrain the flow of free energy, thereby performing work. Processual accounts, and Winning and Bechtel's characterization of mechanisms, concur on the importance of flow, specifically in the case of Winning and Bechtel, the flow of free energy.

Given that both these mechanists and the processual theorists appeal to flow, where does the disagreement arise? Mechanistic accounts also invoke constraints—structures that restrict the flow of free energy. All new mechanist theorists appeal to structures or entities. Machamer et al. are explicitly dualists, appealing to both entities and activities as metaphysically basic. While Winning and Bechtel seek to explain activities, they too are dualists, embracing constraints and free energy. A major focus of processual theorists is to argue against entities, viewing entities as unchanging. But entities for mechanists undergo change. Even in the machines human build, the parts are constructed, transformed as the machine operates, and often taken apart when no longer needed.

While the entities of mechanisms are recognized as changing, they are viewed as enduring at the timescale of the operation of a mechanism. In the case of molecular motors, the motor molecules are viewed as enduing entities over the timescale at which the motor is operating. Even as enduring at that timescale, motor molecules undergo change—during the powerstroke, the conformation of myosin changes, resulting in the movement of the lever arm. Going to a shorter timescale, one identifies different states of the molecule—the ATP binding pocket is in one conformation to initiate hydrolysis, which then exerts force that changes the conformation. On mechanistic accounts, entities are not fixed—but are metastable. That is, they are stable at the timescale they are performing their constraint function. The effect of exercising

that constraining role results in change, including change to the constraints themselves. Considerations of timescale are also critical for understanding flow. If one wants to understand activities in the universe, it does not suffice to note that flow or change is universal. One must differentiate flows. Both powerstroke and Brownian motion accounts of the movement of molecular motors need to distinguish different processes of flow—different changes as myosin moves along actin. The flow of thermal energy or Gibbs free energy is constrained differently at different steps in the interaction of myosin with actin. Scientists appeal to mechanisms to explain how myosin constrains the flow of energy to carry out the activities of muscle contraction.

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Chapter 5, in full, is a reprint of the material as it appears in Bollhagen, A. & Bechtel, B (under review).