What Sustains Life?

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Consilient Mechanisms for Protein-Based Machines and Materials

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Springer

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To my mentor, Henry Eyring, whom I yet strive to represent well,

To my parents for their striving to instill values and a strong work ethic,

To my wife, Kathleen, for her support enabling me to practice the work ethic required by this vocation,

To my children—Kelley, David, Douglas and Weston—for all they have taught,

To the Office of Naval Research and Program Officers, Michael Marron and Keith Ward, for the financial and other support that provided the foundation for the research on which this volume is based.

Preface

In the sixth century BC, Thales of Miletus, father of the Ionian Enlightenment, setting aside the mythic views of Homer and Hesiod asked, "What is the world made of?" and thereby became the first physicist.[1] He answered that water is the basis of all matter and thereupon became an often cited example of early Greek reasoning gone astray. However, not only did Thales initiate scientific inquiry, but also, with reference to living things, he was substantially correct. Living organisms are composed mostly of water, but the unique role of water in living organisms has been wanting for adequate description. From the perspective of D.H. Lawrence, "Water is H_2O, hydrogen two parts, oxygen one part, but there is a third thing that makes it water and nobody knows what that is." From our perspective and as advanced in this volume, the interaction of water with dissimilar groups comprising each protein molecule, the competition for water between these disparate substituents along chain molecules of living organisms, and the freedom of motion that water gives protein chains combine to provide the physical basis of Life.

This book, *What Sustains Life? Consilient Mechanisms*[2] *for Protein-Based Machines and Materials*, at its foundation is a monograph. It arises from the design of elastic-contractile model proteins to convert energy from one form to another and thereby constitutes a limited element of natural history. Such a seemingly specialized focus, however, unfolds to present insight into how living organisms access energy in the environment to become thriving entities of progressively increasing complexity and diversity. Thus, it becomes an account of the means whereby Life can flourish as a physical–chemical entity. In addition, this book unfolds a journey of personal Ionian Enchantment,[3,4] for it contains elements of a memoir. The content draws principally upon three decades of collaborative research and its analysis; it recounts the original design and demonstration of model proteins interconverting the set of six energies interconverted by living organisms, and it arrives at interlinked pervasive consilient mechanisms.

Quite apart from one's theological perspective, a general fascination exists with the physical basis of Life. On the one hand, a living organism seems so improbable and so fragile, and yet, on the other hand, Life can seem so hardy and resilient. Living organisms are complex blends of interdependent and cooperative molecular machines with an innate facility to evolve toward greater complexity of structure and diversity of function. What are the physical forces and mechanisms whereby Life can exist? In our view, at its foundation is a competition for hydration between disparate chemical groups distributed along a protein chain and a resultant phase separation of oil-like components of proteins from water.

Our view of "What Sustains Life?" allows for a relatively nontechnical level of description. At the start, the perspective presented here does not require complex mathematical analyses and specialization. Rather, the concepts grow from commonplace experience such as noting the immiscibility of oil and water or, perhaps even more appropriately, the separability of oil from vinegar. Accordingly, an effort has been made, within the author's near icarian[5] tendencies and other limitations, to reach a broader audience. Should interest develop, original publications and more in-depth analyses within Chapter 5 can be sought for greater detail. Also a more in-depth volume is under development through presentations of a graduate course of 24 lecture hours on contractile protein-based machines and materials.[6]

Chapter 1 introduces the message of the book in terms of four assertions. The first assertion, the phenomenological assertion, derives from the design of a series of model proteins capable of converting the family of energies interconverted by living organisms by controlling association of oil-like domains. A set of five axioms result from the phenomenological assertion. The second assertion, the mechanistic assertion, presents a single molecular process of association of oil-like domains whereby model proteins can function as machines capable of accessing available energies and converting them to those energies utilized in sustaining Life. An elemental understanding of elasticity couples with the association of oil-like domains to complete the second assertion. The third assertion, treated in Chapters 7 and 8, refers to the wider biological literature and presents argument for the dominance of a common unifying mechanism in the functioning of proteins, the molecular machines of Life. Chapter 7 contains examples such as oxygen transport, blood clotting, formation of the amyloid deposits of Alzheimer's disease, and the protein-based machine, chaperonin, that unfolds and refolds proteins having inappropriate association of oil-like domains. Chapter 8 provides an example for each of the three fundamental energy conversions of living organisms: (1) electron transfer that concentrates acid (protons) on one side of the inner mitochondrial membrane with Complex III of the electron transport chain as the enlightening example, (2) the use of the protons by the vital

rotary motor called ATP synthase to produce ATP (adenosine triphosphate), the energy currency of biology, and (3) protein motors that use ATP as their energy source to perform the work of the cell. The prominent example of the latter features the linear motor of muscle contraction that requires ATP as its energy source. The common underlying pair of physical processes that provide for the disparate energy conversions have been named consilient mechanisms in that they "create a common groundwork of explanation," which E.O. Wilson gives as the definition of consilience.[3] Accordingly, the new insight into energy conversion provides interlinked consilient mechanisms[7] for the diverse energy conversions that sustain and define Life.

The thesis of the third assertion begins, "*Biology thrives near a movable cusp of insolubility*," and, as one of its hallmarks, describes ATP production by ATP synthase as the result of a three step rotary motor having as one of the three sides of its rotor an insoluble face that rotates from the insolubility of oil-like association with sleeve to solubility due to repulsion with the sleeve containing vinegar-like precursors, ADP (adenosine diphosphate) and P_i (inorganic phosphate), to drive formation of ATP, the universal energy coin of biology. Analogous effects occur in ATPases, the molecular machines that use ATP as their energy source. In muscle contraction the binding of ATP and its breakdown to ADP and P_i converts insoluble domains of the contracted state to soluble domains, and their release returns oil-like domains to the insoluble contracted state to drive linear motors.

The fourth assertion applies the understanding of the new mechanism in the development of protein-based materials to improve health care, to decrease healthcare costs, and to assist in alleviating additional major problems of society.

Chapter 2 presents an overview of the energy conversions that sustain Life and steps further toward the molecular processes involved. Chapter 3 notes highlights of the grand, yet at its outset proscribed, pilgrimage that has given us an understanding of components and products of living things. The enigma of Life erodes further as the premier, yet erstwhile improbable, molecular machines of Life, the proteins, emerge as described in Chapter 4, to be almost energetically wasteful in construction. With knowledge of the biochemical details of protein synthesis,[8] the protein molecule transforms from a highly improbable entity to an example of extravagant expenditure of energy in the process of construction. Significantly, however, protein machines exhibit remarkable efficiency in function, and it is an energy-fed march toward more diverse and efficient molecular machines that constitutes the basis for evolution through natural selection.

Chapter 5 is the scientific core of the book. It begins with familiar phase transitions of ice-to-water and water-to-vapor, both of which demonstrate increased disorder with increased temperature. It then brings in the unique phase transitions of the two-component system, model proteins in water, in which the protein

component progresses to increased order and the water component to decreased order with increased temperature. Next, it experimentally derives five axioms for protein function as molecular machines by means of designed model proteins. This chapter describes the development of diverse molecular machines, utilizing a single, consilient mechanism to achieve diverse function. The approach begins with a particularly compliant molecular building block. Rules are demonstrated whereby this building block of elastic model protein can be made to perform mechanical work simply by heating, and then mechanical and a number of other forms of work performed by living organisms can be made to occur at physiological temperature. Herein lies your author's personal Ionian Enchantment.

Following the historical development, initially, contraction to lift a weight is demonstrated by heating cross-linked elastic model protein. Shortly thereafter this performance of mechanical work is demonstrated without the use of thermal energy, but instead with the chemical energy of salt on the parent model protein and then with acid acting on a designed modification of the initial model protein. Additional model proteins are designed, each utilizing a common underlying mechanism, and found capable of the many different energy conversions extant in biology. Each designed model protein, once constructed and tested, functioned as intended in diverse energy conversions.

Reversing the trend to examine smaller protein components, a particularly compliant model protein system had been chosen to which new functional capacities could be added. It has been a simple exercise of the scientific method with specific elements of an axiomatic approach of Bacon with awareness of the illusion of knowledge,[9] of Descartes with development of graphical methods[10,11] of Galileo with experimental exploration and verification,[12,13] and of so many others. In particular, temperature-induced oil-like aggregation of model proteins gave rise to a hypothesis. The hypothesis was tested by sequential construction of modified model proteins and followed by experimental verification. There was simple extension of a basic concept and its translation into new model protein constructs. The result was development of protein-based machines for diverse energy conversions not previously demonstrated in model proteins, and the family of energy conversions obtained were those found in living organisms. Experimental testing directly followed, with the result that the new model protein constructs worked as expected, each capable of a different type of energy conversion and, within a type, capable of different efficiencies for energy conversion. The common underlying mechanism for all of the energy conversions, the consilient mechanism, was found to be competition for hydration between oil-like and vinegar-like groups constrained to coexist along the protein chain molecule that exhibits as a repulsion between oil-like and vinegar-like groups.

The molecular players in the drama of sustaining Life are introduced in Chapter 3, the biosynthetic process of protein construction is explained in Chapter 4, and the diverse energy conversions and their mechanism are demonstrated in Chapter 5. With this background, we can now address the question of the evolution of protein machines. In Chapter 6, we respond to the challenge of Behe: "if you search the scientific literature on evolution, and if you focus your search on the question of how molecular machines—the basis of Life—developed, you find an eerie and complete silence."[14] The silence that Behe reports is borne of a lack of awareness of the broader scientific literature, which is not silent on the issue of how molecular machines can evolve. As part of our thesis here, the literature reviewed in Chapter 5 contains experimental demonstration of *de novo* designed protein-based machines of increasing diversity, complexity, and efficiency. Given the consilient mechanism of energy conversion detailed in Chapter 5, the evolution of protein-based molecular machines unfolds as a remarkably simple process. Chapter 6 demonstrates how an elementary mutation, a single base change, allows a thermally driven protein machine to become a chemically driven machine, that is, to access a new energy source. Another single base change can allow access to yet another source of energy. In fact, each new energy source in the set of energy conversions of living organisms demonstrable by these designed model proteins is accessible by a single base change. Furthermore, in each case a subsequent single base change results in a more efficient molecular machine. Because of this simple access to new energy sources with greater efficiencies, each step toward more diverse and efficient protein-based machines occurs without an additional cost to the organism to produce. Evolution toward more efficient and more complex protein machines becomes a natural step-by-step process when viewed in terms of the consilient mechanism of energy conversion and in terms of the genetic code and protein biosynthesis. In the simplest case, one might consider two organisms each requiring the same amount of food, one to produce a less efficient and less effective machine and the other to produce a more useful and more efficient machine. Because both less-efficient and more efficient protein-based machines cost the same amount of energy to produce, in the competition for survival the improved organism wins. This provides elementary insight imto natural selection.

Chapter 7 begins with the assertion that biology thrives near a movable cusp of insolubility and that excursions too far either direction into the realm of insolubility or of solubility spells disease and death. For example, sickle cell disease represents an excursion too far into the realm of insolubility, as do the more ominous prion diseases of Alzheimer's disease, and mad cow disease. Blood clotting is a special example of thriving at the cusp of insolubility. This chapter also discusses molecular chaperones

whose role is to bring proteins back from the realm of incorrect insolubility and ends with a brief example of shifts toward excess solubility due to oxidative processes with the result of degradation and disease such as pulmonary emphysema.

Chapter 8 continues with the thesis that biology thrives near a movable cusp of insolubility whereby the forces that, in a positively cooperative manner, power the molecular machines of biology drive spatially localized oil-like regions of protein back and forth across movable water solubility–insolubility divides, that is, back and forth between being associated (water insoluble) and dissociated (water soluble). Life's commonplace energy source is ATP. Life's workhorse protein-based machines are ATPases, where ATP binding and its breakdown to ADP and P_i acts like cocking of a gun and P_i release functions like the discharging of the gun. Binding ATP, and especially its breakdown to ADP and P_i, moves the cusp of insolubility, the water solubility–insolubility divide, to solubility, and the release of P_i functional components (hydrophobic domains) of the machine to insolubility. Remarkably, even one part of the two-part rotary protein machine that produces ATP from ADP and P_i functions in reverse as an ATPase that breaks ATP down to ADP and P_i to perform work with high efficiency.

The primary examples in Chapter 8 represent three key aspects of energy conversion in animals. The first explicitly described protein-based machine pumps protons across a membrane to create acid on one side of the membrane; it is Complex III of the electron transport chain of mitochondria, the energy factory of the cell. The second protein-based machine uses the return of the protons back across the membrane to make ATP, the energy coin of living organisms; it is ATP synthase, as noted immediately above. The third protein-based machine uses the ATP to produce motion; it is the myosin II motor of muscle contraction, categorized as an ATPase. The latter presents a transparent demonstration of the consilient mechanism whereby binding ATP (adenosine tri*phosphate*) disrupts association of oil-like domains and loss of *phosphate* re-establishes association of the oil-like domains to provide the power stroke of a contraction. In addition, further scrutiny of motion-producing protein-based machines couples the cusp of insolubility aspect of the consilient mechanism to a "common groundwork of explanation" of an increase in elastic force resulting from a decrease in motion along the backbone of a single protein chain. In doing so, this sets aside a popular concept of polymer elasticity that required random chain networks and a Gaussian distribution of end-to-end chain lengths (See Appendix 1).

In Chapter 9, the capacity to design protein-based molecular machines and materials turns toward the development of useful applications for improving individual health and environmental health. With insight as to how the protein-based machines of biology work, and, because we know how they work, we can

design materials for the future as never before! A principal medical application considered here employs a consilient approach to tissue engineering; it utilizes materials and mechanisms natural to the tissue to be restored or augmented and couples these properties to the natural capacity of cells to function, for example, as mechano-chemical transducers. This area involves such specific health concerns as prevention of urinary incontinence, prevention of postsurgical adhesions, intervertebral disk restoration, and temporary functional vascular and urological scaffoldings with the potential to remodel into natural tissues.

Another general medical application with great promise is the area of drug delivery also referred to as the controlled release of pharmaceuticals, including natural proteins and genes. Specific areas under development include transdermal delivery for the prevention of pressure ulcers, drug addiction intervention, programmed adjuvantcy and release of vaccines, and analgesics and anesthetics. Although there are numerous nonmedical applications, the development of programmably biodegradable plastics, of specific transducers and biosensors, and of new sound-absorbing materials, among others, are addressed.

After the more technical aspects of the book are integrated, the Epilogue places the message of the book into broader context. It considers Schrödinger's *What Is Life?*[15] and the Prigogine argument for biology functioning as creating order out of chaos under far-from-equilibrium conditions. It speaks to the oft-noted paradox of time's arrow for the universe pointing toward less order and uniformity, whereas time's arrow for Life and Society points toward increasing structure and diversity. In short, explicit examples of protein machines are given whereby living organisms utilize products of photosynthesis to create structure by means of a pair of efficient consilient mechanisms.

Thus, life is a complex integration of mutually dependent protein machines with a unique energy-driven capacity to reverse time's arrow for the wider universe. Life originated and continually evolves by employment of machines, composed of large protein polymers, to capture available energy and by use of the energy in many small, not far-from-equilibrium steps to create the structures of Life. Life is not well represented by transient dissipative structures like tornadoes. Rather, Life provides examples of seeds and spores that can lie dormant for years and even centuries only to spring to life on receiving the proper modest energy inputs. We conclude this molecular machine perspective of Life with an apparent message. Time's arrow for Life and for Society points toward greater complexity of organization and diversity of function only as long as there remain adequate energy sources.

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2006

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3. E.O. Wilson, *Consilience, The Unity of Knowledge*, Alfred E. Knopf, New York, 1998, pp. 4–5. On these pages Wilson describes his youth in southern Alabama as being immersed in the rich fauna and flora, principally insects, of the region. On introduction at the University of Alabama in Tuscaloosa, Alabama to the concept of Darwinian evolution as advanced by Ernst Mayr's 1942 *Systematics and the Origin of the Species*, Wilson describes the excitement of his own unfolding Ionian Enchantment as he became aware of the relationships among the many species of his personal experience.
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The ever-challenging, original editor for this volume, Alla Margolina-Litvin stimulated discussions that lead to the broader scope of the present volume rather than to a more staid volume on the engineering of protein-based machines and materials. Alla's challenge was to write a follow up to the Schrödinger book, *What Is Life?*, of six decades ago. Drawing from the recognition that our deigned elastic-protein-based polymers could interconvert the energies interconverted by living organisms and that accessing energy in the environment was the key to sustaining Life, I believed that the present title was warranted. This set a path of developing an analytical approach of the data on our elastic-contractile model proteins in order to considering more explicitly the protein-based machines of biology and a path to the broader implications of the discovery of energy conversion by the inverse temperature transition. Attempting to deliver a manuscript worthy of the title has taken much longer than originally planned and includes new data and analyses.

Many coworkers contributed over the past several decades during which the foundation for the consilient mechanisms developed. The many inadvertent shortcomings within the book itself should be recognized as mine rather than theirs.

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2006

Contents

1
Introduction

1.1 About the Title

1.1.1 What Sustains Life?

An account of *what sustains Life* is an effort more modest in scope than either an attempt to explain *what is Life*[1] or a challenge to recount *where or how Life began.*[2] The latter focus more on heredity and address the transition from a prebiotic to a biotic earth, which concern the more profound transition from the absence to the presence of self-sustaining, self-replicating Life. With present knowledge it seems possible that the origins of Life could have involved far-from-equilibrium conditions of the Prigogine focus.[3]

From the work of many gifted biochemists, however, we do know that the creation of the chain molecules of the living organism, the nucleic acids and proteins required to duplicate and sustain Life, does not, as has been proposed,[3] require far-from-equilibrium conditions and is not the result of dramatic dissipative processes. Building of Life's great molecules of heredity, the nucleic acids, occurs by means of one reversible small energy step after another, but with a special trick. The translation of these nucleic acid sequences into protein sequence also occurs, one reversible small energy step after another, but again with the special twist. As presented in Chapter 4, the relentless drive toward these otherwise improbable macromolecules of Life derives simply from the enzymatic removal of a reaction side product. Removal of a reaction product blocks reversal of chain growth. Each individual reaction required in the process of adding each nucleic acid residue to form the polynucleotides of DNA and RNA, or of adding each amino acid residue to form protein of hundreds, and even thousands, of residues, represents a small energy step of discarding some 8 kcal/mol reaction. Nonetheless, in sum, with the repeated discarding of the energy within a reaction product, the production of a protein represents an enormous expenditure of energy that, once recognized, removes any sense of protein improbability.

Except for the singular events of the absorption or emission of a photon of light, the energy conversions that sustain Life occur by relatively small energy steps, one-tenth the magnitude of the energy of a single initiating photon from the sun. The present effort, however, begins with the living organism and seeks to describe how these relatively small steps in energy sustain Life and allow for continual evolution into more diverse, complex, and efficient entities.

1.1.2 Access to Energies That Sustain Life Derives from a Special Phase Separation Exhibited by Proteins

Having temporarily addressed the question of how Life's great molecules come into existence, we address the question posed in the title to this book. The answer necessarily resides in the underlying function of these remarkable macromolecules. The following two statements, each derived from its subsequent bulleted train

of thought provide a phenomenological glimpse into our perspective of biomolecular functions that sustains Life.

Accessing energy from the environment sustains Life!

- What makes it possible for Life to exist?
- No biological problem is more profound!
- Yet, at one level, the answer is quite simple and even well known.
- It is the capacity to utilize energy sources, for example, food, in the environment!

A phase separation process accesses available energy!

- The above answer, however, defers the currently challenging question.
- What converts available energy in food and in oxygen to energies essential for Life?
- As presented here, the answer again becomes quite simple!
- Protein-based machines undergo a special kind of *phase separation* that converts energy from one form to another!

1.1.3 A Consilient Mechanism: A Unifying Thesis for *What Sustains Life*?

1.1.3.1 Thesis

Biology thrives near a movable cusp of insolubility, and the forces that, in a positively cooperative manner, power the molecular machines of biology drive paired oil-like domains of proteins back and forth between association (insolubility) and dissociation (solubility), and excursions too far in either direction into the realms of insolubility or solubility spell disease and death.

1.1.3.2 Recognition of a Consilient Mechanism

Whether the function of a molecular machine is to produce motion or convert one form of chemical energy into another or to perform any of several other energy conversion functions of biology and whether dysfunction occurs that results in disease and/or death, in our view, there exists a "common groundwork of explanation,"[4] that is, there occurs a consilient mechanism.[5] From our perspective, the process of phase separation from water by association of oil-like domains, that is, of insolubilization, provides a consilient mechanism for diverse energy conversions that sustain Life.

As demonstrated by elastic-contractile model proteins, the consilient mechanism achieves essentially all of the energy conversions that sustain Life. Knowledge of the mechanism arose *not* from analysis and extension of a particular biological energy-converting system, such as that of muscle contraction. Instead, the mechanism originated by *de novo* design, by designing entirely new energy-converting functions into a component of the mammalian elastic fiber never known or previously considered for such functions. On the contrary, the role of the mammalian elastic fiber is to store the energy of deformation and to use the stored energy to restore the nondeformed state as the deforming force recedes.

1.1.3.3 Oil-Like Groups Are Primarily Hydrocarbons

The most representative oil-like groups are the elemental hydrocarbon units, $-CH_2-CH_2-$, and $=CH-$; these are the most common chemical groupings of lubricating oils, of gasoline and heating oils and gases, and, of course, of biological fats and lipids. When these hydrocarbons combine to form the side chains, the R-groups, of a protein chain molecule, the protein with a balanced occurrence of these side chains is soluble in water at low temperature, but on raising the temperature these oil-like groups associate, that is, they become insoluble. There is a particular *temperature interval* over which insolubility develops. For reasons considered in detail in Chapter 5, this transition from solubility to insolubility is called an *inverse temperature transition*.

1.1.3.4 Definition of the Cusp of Insolubility

For our model elastic-contractile proteins in water, a plot of heat absorbed on increasing temperature exhibits an abrupt rise and then a more gradual decline as the temperature reaches the start of and passes through the tran-

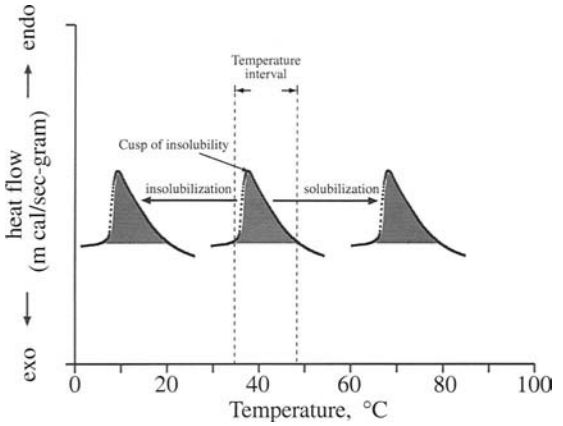

FIGURE 1.1. Schematic representation of the *movable cusp of insolubility* based on actual experimental data of the heat absorbed as the temperature is raised through the range of the phase separation for association of oil-like domains within an elastic model protein. Starting at the tip of the cusp and tracing to lower temperatures gives solubility of oil-like groups, seen as swelling within a cross-linked matrix. On the other hand, tracing to higher temperatures gives insolubility of oil-like groups, seen as contraction for a cross-linked matrix and the capacity to *pump iron*. Additionally, instead of changing the temperature, many different energy inputs can move the cusp to lower temperatures to drive contraction or can move the cusp to higher temperatures to cause relaxation (swelling).

sition to insolubility. As shown in Figure 1.1, the trace of the curve looks like the cross section of an incisor, of a cuspid. The trace presents a *cusp of insolubility*.

As defined in *Webster's Third New International Dictionary*, a cusp is "a fixed point on a mathematical curve (graph) at which a point tracing the curve would exactly reverse its direction, that is, a point at which traversing in opposite directions has opposite consequences."[6] Accordingly, starting from the peak of the curve and going to lower temperatures gives solubility, whereas starting from the peak of the curve and going to higher temperatures gives the opposite consequence of insolubility. As is also discussed in Chapters 5, 7, and 8, every energy input of relevance to biology, other than heating itself to surmount the cusp, moves the location of the *cusp of insolubility* along the temperature axis either to lower or higher temperatures. Hence, the phase separation of oil-like domains from water behaves as a *movable cusp of insolubility*. As shown in Figure 1.1, energy inputs that lower the temperature range of the *cusp*, that is, that lower the onset temperature for the phase separation, cause insolubility, and energy inputs that raise the onset temperature for the phase separation result in solubility. In other words, the *cusp of insolubility* simply marks the movable boundary between solubility and insolubility of an oil-like domain, a cluster of oil-like side chains, of a protein.

1.1.3.5 Excursions Too Far from the Solubility–Insolubility Boundary

The plaques of Alzheimer's disease and the fibrous state of the prions of mad cow disease (both with resulting brain destruction), the thrombi of stroke (cerebral thrombosis) and of heart attack (myocardial infarction), and the familiar manifestation of death (rigor mortis) represent excursions too far in the direction of protein insolubility. The favorable actions of antioxidants keep proteins from becoming so soluble (unfolded) that protein function disappears and proteolytic degradation ensues. Of course, the lack of blood clotting, hemophilia (the lack of clotting proteins to become insoluble by association of oil-like domains), results in death. Such devastations result from loss of proper balance between solubility and insolubility. They represent excursions too far from the *cusp of insolubility*, that is, too far from the boundary between insolubility and solubility.

1.1.3.6 Excursions Back and Forth Across the Solubility–Insolubility Boundary

1.1.3.6.1 Linear Motors of Biology

As is argued in Chapter 8, small, often reversible, energy excursions back and forth across the boundary between associated (water-insoluble) and dissociated (water-soluble) oil-like domains (clusters of oil-like groups) drive the protein-based machines of biology. Biology often achieves mobility by many linear motors comprised of protein, such

as represented by muscle. In our view, oil-like regions, covered by organized water within motion-producing components of a protein motor, associate on loss of charge with release of water. The association of oil-like domains stretches interconnecting dynamic chain segments. This results in an increase in entropic elastic force due to a decrease in internal chain motions.[7] The entropic elastic force, thus developed, converts to motion as the extended chain retracts and the internal chain motions of interconnecting chain segments regain the increased amplitudes of the relaxed state.

1.1.3.6.2 Rotary Motors of Biology

For rotary motors within biological membranes, the oil-like domains of the rotor function much like special cleats on the rim of a tractor wheel passing into the oil-like domain of the membrane sleeve. In short, oil-like domains associate on loss of charge (on loss of vinegar-like species). Association of oil-like groups represents a *pull* component of force. There also, however, occurs a *push* component of force in the repulsion between oil-like and vinegar-like groups in the coupled extramembrane component of the rotary motor that produces almost 90% of biology's energy currency in the form of ATP (adenosine triphosphate). In particular, the oil-like domains either become repulsed by the presence of charge or, when externally forced to confront charge, repulse charge and force the charged entity to become less charged. As is introduced below and developed in Chapters 5 and 8, the latter is proposed to cause the most charged state, bound ADP (adenosine diphosphate) plus phosphate, of biology's ATP synthase rotary motor to combine to become a less charged ATP, the energy coin of biology. This production of ATP, in our view, occurs through the combination of *push* and *pull*.

1.1.4 Protein-based Machines Push and Pull!

The consilient mechanisms in relation to protein-based machines of biology are introduced. Before the four major assertions that form the basis of this book are elaborated, the next paragraphs build a mental framework for further elements associated with the rejoinder to the question posed in the title.

1.1.4.1 Elemental Contractile Event as One Aspect of Protein-based Machines

A change in the three-dimensional shape of a chain-like molecule necessarily changes the distance between two of the repeating units (or links) forming the chain molecule. If one of the two units is fixed in space and the other is attached to a weight, then the change in shape of the chain-like molecule moves the weight. Proteins are chain molecules where each link is decorated by any one of twenty different chemical side groups with each link derived from any one of twenty different amino acids. Accordingly, the change in shape of a protein represents an elemental contractile event whereby an object could be moved, and there can be *pull* and *push* elements to the mechanical event. Because of the many different repeating units available and because of the absolute control of their sequence in the protein chain, there are many ways with which to achieve the change in shape. Immediately below, four lists state the arguments for the roles of contractile proteins in biology.

1.1.4.2 Contractile Machines That Perform Mechanical Work by Pulling

- Machines perform useful work by converting energy from one form or location to another.
- Energy inputs cause shape changes in protein-based machines because of changes in association of oil-like domains.
- Such shape changes cause contractile protein-based machines to perform mechanical work.
- By this means, contractile protein-based machines can lift or pull weights; they can *pump iron.*

1.1.4.3 Contractile Linear Motors of Protein-based Machines

- The protein chain of a linear motor may be attached at both ends.

- Association of oil-like groups of certain chain segments between attachments stretch other interconnecting chain segments.
- On stretching (pulling), the interconnecting chain segments develop elastic force.
- The stretched interconnecting chain segments either retract and pull the attachment sites closer together or increase the force sustained at immovable attachment sites.

1.1.4.4 Contractile (Push–Pull) Rotary Motors Using Acid to Produce ATP

- Changing oil-like associations between fixed sleeve and rotary components drives rotation, whether intramembrane or in an attached extramembrane location.
- Reduction of charge in a transmembrane cleat of an otherwise oil-like *rotor* rotates a newly formed oil-like cleat into the cell membrane's sea of oil.
- By rotation, the most oil-like side of the extramembrane rotor faces off through a cleft of water with the most charged state of sleeve to create maximal (oil-like–vinegar-like) repulsion.
- The most charged state, ADP+P, relaxes repulsion (the push component) coming from the most oil-like side of the rotor by forming less-charged ATP.

1.1.4.5 Contractile Machines That Perform Work Other Than Mechanical

- The contractile protein machines of Life, however, accomplish more than the mechanical work of *pumping iron* or of rotation.
- By proper choice of sequence, they can pump protons; they can perform chemical work.
- By further design variation, they can pump electrons; they can perform electrical work.
- In the process of contracting, they can perform even additional kinds of work essential for Life.

Thus, the perspective is that contractile parts of protein-based machines sustain Life.

1.1.5 Protein-based Materials!

Contractile protein-based machines become useful materials:

- Knowledge of how contractile protein machines can sustain Life provides the capacity to design protein-based materials.
- Designed and synthesized protein-based materials have the potential to restore and sustain individual health and societal health.
- Designed contractile protein materials can perform functions beyond that which evolution has called upon them to do.
- They can be designed to deliver drugs, to prevent postsurgical adhesions, to restore diseased tissue, and to be environmentally friendly biodegradable thermoplastics.

Accordingly, protein-based materials hold promise of restoring and sustaining individual and societal health.

1.2 Four Principal Assertions of "What Sustains Life?"

This book stands on four principal assertions:

1. The phenomenological assertion
2. The mechanistic assertion
3. The assertion of biological relevance
4. The applications assertion

Introductions to these assertions follow.

1.2.1 Assertion 1: The Phenomenological Assertion

Designed elastic model proteins exhibit diverse functions that mimic biological functions by diverse means of controlling association of oil-like domains. As a result, five experimentally derived axioms phenomenologically categorize means by which energy conversions occur through control of association of oil-like domains.

A diverse set of energy conversions that sustain life can be experimentally demonstrated by *de novo* design of elastic-contractile model proteins under the precept of a single, pervasive, mechanism, that is, by a consilient mechanism that "creates a common groundwork of explanation."[4,5] It is a mechanism that achieves function by controlling association of

oil-like domains in an environment of water. Five experimentally based axioms characterize the phase separation process of an inverse temperature transition for association of oil-like domains (see Chapter 5, Section 5.6.3). The axioms provide a phenomenological foundation with which to begin an understanding of protein function and with which to design protein-based machines and materials.

Phase separation occurs as oil-like groups separate from water!

- What is the nature of the *phase separation*?
- The answer lies within the repulsive energies responsible for the adage that "oil and vinegar don't mix"!
- Oil-like and vinegar-like adornments, constrained to coexist along the protein chain, cannot similarly separate.
- Instead, separation occurs by chain folding and assembly whereby the oil-like groups associate, separate from vinegar-like groups, and close off from water!

1.2.2 Assertion 2: The Mechanistic Assertion

1.2.2.1 With the Proper Balance of Oil-like Groups and Charged (Vinegar-like) Groups, There Exists a Competition for Limited Water Between Oil-like and Vinegar-like Groups Constrained to Coexist Along a Protein Chain

Development of too much structured water around emerging oil-like groups causes oil-like domains to reassociate. Decrease of organized water surrounding newly emerged oil-like groups, as charged groups successfully compete to add water to their own hydration shells, causes oil-like domains to dissociate. The energy change represented by association, or dissociation, of oil-like domains can be quantified using the information of the heat change represented by the cusp of insolubility in Figure 1.1.

These concepts of mechanism, developed in Chapter 5, are introduced immediately below by two statements, each followed by a list that develops the statement.

1.2.2.1.1 The Development of Too Much Organized Water Surrounding Oil-like Groups Causes Phase Separation!

- Vinegar-like groups, often as ions, prefer being surrounded by organized water.
- Oil-like groups, however, become surrounded by differently organized water, unsuited for ions.
- Development of too much water organized around oil-like domains renders them insoluble, and they associate, that is, separate from water.
- To control the association of oil-like domains is to control protein function and to allow for design of remarkably efficient and diverse energy conversions.

1.2.2.1.2 The Competition for Water Between Oil-like and Vinegar-like Groups Controls Separation!

- Charged vinegar-like groups steal organized water from around emergent neighboring oil-like groups and allow solubility of otherwise insoluble oil-like domains.
- Abundant structured water surrounding oil-like groups can force neutralization of charged groups and allow protein folding and assembly by association of oil-like domains.
- In addition to contraction by association of oil-like domains, such forced neutralization results in the performance of chemical and electrical work that sustains Life.
- More intense competition for water between oil-like and charged groups yields more positive cooperativity and correspondingly more efficient energy conversion.

1.2.2.1.3 Properties Once Considered Unique and Essential to Life Are Unexpectedly Found in Designed Model Proteins

The particular designed elastic model proteins, through which the mechanistic assertion developed, use a repeating five-amino-acid residue sequence propagated by *translational symmetry*. Of the five axioms noted above under the phenomenological assertion, the fifth axiom describes the conditions for increased positive cooperativity and the result of increased effi-

ciency. Remarkably, positive cooperativities exhibited by these designed elastic model proteins, at their best, markedly exceed generally discussed biological examples of positive cooperativity. This was unforeseen.

As Monod states in his treatise, *On Symmetry and Function in Biological Systems*, "One may set aside the simple problem of fibrous proteins. Being used as scaffolding, shrouds or halyards, they fulfill these requirements by adopting relatively simple types of *translational symmetries*."[8] Therefore, it was not anticipated that positive cooperativity, the effect Monod thought to be "the second secret of life," second only to "the structure of DNA,"[9] would be most beautifully demonstrated by designed variations of a repeating sequence of the mammalian elastic fiber based on translational symmetry.

Based on a series of designed elastic-contractile model proteins, Figure 1.2 exhibits a family of curves whereby stepwise linear increases in oil-like character give rise to supralinear increases in curve steepness, that is, in positive cooperativity. More oil-like phenylalanine (Phe, F) residues with the side chain $-CH_2-C_6H_5$ replace less oil-like valine (Val, V) residues with the side chain $-CH-(CH_3)_2$. Here the structural symmetry is translational with as many as 42 repeats (Model protein v) of the basic 30-residue sequence, and the structure is designed beginning with a repeating five-residue sequence of a fibrous protein, the mammalian elastic fiber.

Figure 1.2 demonstrates a series of increasingly shifted and increasingly steep acid–base titration curves that correspond with linear

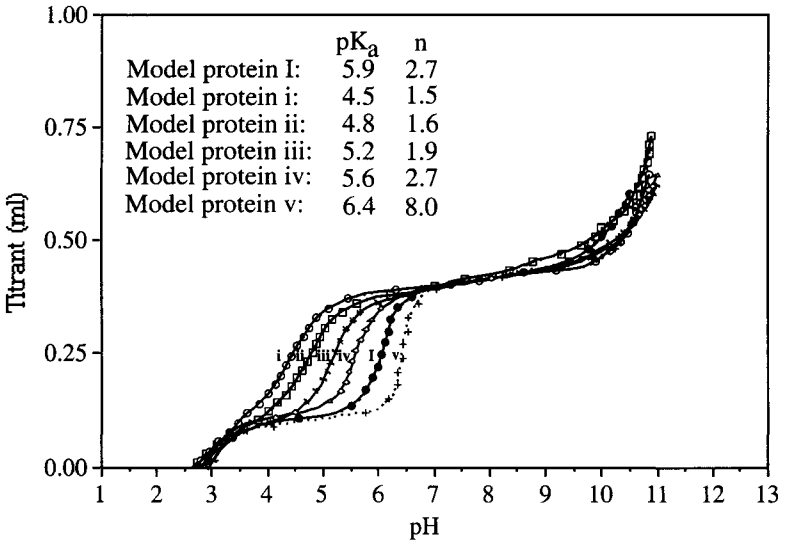

FIGURE 1.2. Plots of the acid–base titration curves for the following series of model proteins:

Model Protein I:	(GVGIP GFGEP GEGFP GVGVP GFGFP GFGIP)$_{26}$(GVGVP)	2E/5F/2I
Model Protein i:	(GVGVP GVGVP GEGVP GVGVP GVGVP GVGVP)$_{36}$(GVGVP)	E/0F
Model Protein ii:	(GVGVP GVGFP GEGFP GVGVP GVGVP GVGVP)$_{40}$(GVGVP)	E/2F
Model Protein iii:	(GVGVP GVGVP GEGVP GVGVP GVGFP GFGFP)$_{39}$(GVGVP)	E/3F
Model Protein iv:	(GVGVP GVGFP GEGFP GVGVP GVGFP GVGFP)$_{15}$(GVGVP)	E/4F
Model Protein v:	(GVGVP GVGFP GEGFP GVGVP GVGFP GFGFP)$_{42}$(GVGVP)	E/5F

Stepwise increases in oil-like character, as when the mildly oil-like Val (V) residue is replaced by the very oil-like Phe (F) residue, cause the acid–base titration curves to be shifted to higher pH values and to be steeper. The energy required to drive the model protein from the phase separated, contracted state to the swollen, relaxed state is proportional to the width of the curve, that is, inversely proportional to the steepness of the curve. Accordingly, the model protein with the steepest curve exhibits the most efficient function for performing the work of lifting a weight.

stepwise increases in oil-like character of the model proteins. Here the low pH side (the more acidic, uncharged side) of the curve represents the contracted (the oil-like, phase-separated, insoluble) state, and the high pH side is the unfolded soluble state. The energy required to drive contraction is inversely proportional to the steepness of the curve. In particular, a change in pH ($\Delta pH = \Delta G_{CE}/2.3RT$) is proportional to a change in chemical energy, ΔG_{CE}, and, therefore, a smaller change in pH required to go from the relaxed to the contracted state means a more efficient energy conversion. Accordingly, increased steepness signifies more efficient function.

A means of quantifying steepness uses Hill plots, as given in Figure 1.3, where the slope of the Hill plot is the Hill coefficient, n. In the absence of cooperativity the Hill coefficient is 1, whereas the highest positive cooperativity exhibited by the set of elastic-contractile model proteins in Figure 1.3A is 8. As further noted below and shown in Figure 1.3B, the Hill coefficient is 1.0 for myoglobin that binds oxygen in the tissues and 2.8 for hemoglobin, the protein that transports oxygen from the lungs to the

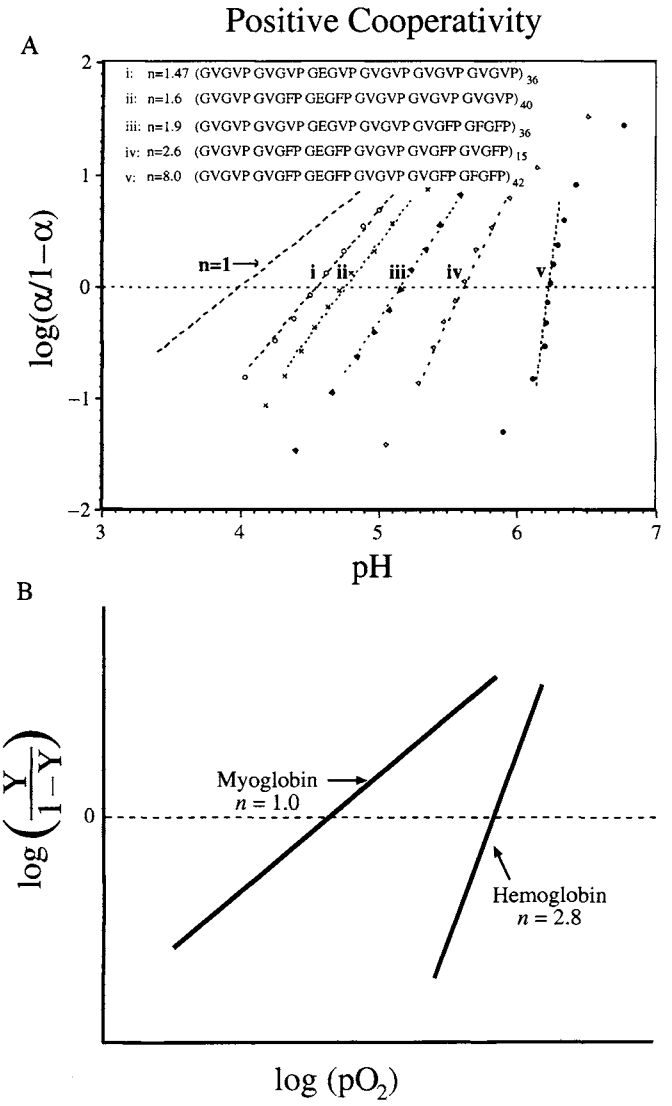

FIGURE 1.3. **A** Hill plot of the set of designed elastic-contractile model proteins shown in Figure 1.2 with Hill coefficients, n, ranging from 1.5 to 8.0. **B** Hill plot of myoglobin (n = 1) and hemoglobin (n = 2.8). It is shown that the vaunted hemoglobin positive cooperativity is relatively small compared with that of designed elastic protein-based polymers and, in particular, of designed Model protein v.

tissues. Myoglobin exemplifies the simple binding of oxygen, whereas hemoglobin is perhaps the most commonly considered example of positive cooperativity in biology, which is essential to its oxygen transport role in biology. The consilient explanation for this difference between myoglobin and hemoglobin is given in Chapter 7, Section 7.2.4.

Most significantly, however, the molecular basis for positive cooperativity and the result of increased functional efficiency in designed elastic-contractile model proteins has been experimentally determined to be the competition for water that occurs between oil-like domains and charged groups constrained to coexist within a protein structure (see immediately below and Chapter 5, section 5.1.7.4). This represents the principal statement of the Mechanistic Assertion.

1.2.2.2 An Explicit Demonstration of the Mechanistic Assertion in Terms of Monod's "Second Secret of Life"

Central to the mechanistic assertion is competition for hydration between oil-like and vinegar-like groups constrained to coexist along a chain molecule. In the process, the disparate groups each reach for water unaffected by the other. This results in an effective repulsion between oil-like and vinegar-like groups, that is, the groups physically get as far away from each other as possible in their thirst for water unaltered by the other. When the sequence does not allow the oil-like and vinegar-like groups to get far enough apart, repulsion exists even in the totally unfolded state. Relaxation of the repulsion occurs when the vinegar-like group becomes less vinegar-like, as could occur on neutralization (e.g., by protonation) or partial neutralization (e.g., by ion pairing) of the charge. As a result, structurally related oil-like groups lose solubility; they develop too much special structured water around them, which becomes the driving force for folding by association of oil-like groups.

The classic vinegar-like group is the carboxylate $-COO^-$, and it becomes less vinegar-like on adding acid, H^+, to become the carboxyl –COOH. The measure of the acid, the pH (= $-\log[H^+]$), required for neutralization of any particular carboxylate is the pKa. This is the pH at which the concentration of carboxylates and carboxyls undergoing change are equal; in other words, $[-COO^-]/[-COOH] = 1$. The pKa is 4.0 or less for glutamic acid (Glu, E) with a side chain of $-CH_2-CH_2-COOH$ or aspartic acid (Asp, D) with a side chain of $-CH_2-COOH$ when not involved in repulsive interactions.

1.2.2.2.1 More Detailed Consideration of the pKa Shifted State Arising from Competition Between Oil-like and Vinegar-like Groups

With the preceding background, Figure 1.4 considers the pKa values relevant to Model protein v using the special way of plotting the data of Figure 1.3. Remarkably, the pKa of the first carboxyl to form carboxylate on raising the pH, on decreasing acid, is 7.0 due to the water-mediated repulsion between oil-like groups and charged carboxylate. As the ionization proceeds, the pKa decreases until the last (the 42nd) carboxyl to form carboxylate of the 42 carboxylates in the chain of 42 repeats of 30 residues does so with a pKa of 5.7. Accordingly, ionization of the last carboxyl becomes more than 20 times more likely than the first one because of the progressive and cooperative destruction of the structured water around exposed oil-like groups.

The repulsion between oil-like groups and charged carboxylates is relaxed by 1.8 kcal/mol-carboxylate as more and more of the special hydration around oil-like groups is disrupted. Formation of the first carboxylate occurs only when it can destructure sufficient structured water around oil-like groups as they become exposed. With the first carboxylate having destructured sufficient structured water around exposed oil-like groups, a subsequent carboxylate with an overlapping sphere of influence can form more readily as it has less structured water around exposed oil-like groups to destructure in order to form its own hydration shell. This constitutes positive cooperativity.

Of perhaps even greater significance in demonstration of the mechanistic assertion is that when the oil-like association is fully disrupted and the carboxylates and the oil-like

groups are fully exposed to water in Model protein v (see Fig. 1.2), there still exists a repulsion between the oil-like and vinegar-like groups that raises the pKa from 4.0 to 5.7. This amounts to a repulsion of 2.4 kcal/mol-carboxylate. *The carboxylates are still unable to achieve full hydration, because they cannot get sufficiently removed from the oil-like groups.* Even with the less oil-like Val (V) residues of Model protein i, a small residual pKa shift remains. When carefully analyzed, the data in Figures 1.2 through 1.4 clearly identify the basis for positive cooperativity and provide an understanding of what Monod has called the "second secret of life."[9] In short, positive cooperativity results from the competition for hydration between oil-like and vinegar-like groups constrained to coexist along a chain of defined sequence and/or within the folded and assembled state of the chain or chains.[10]

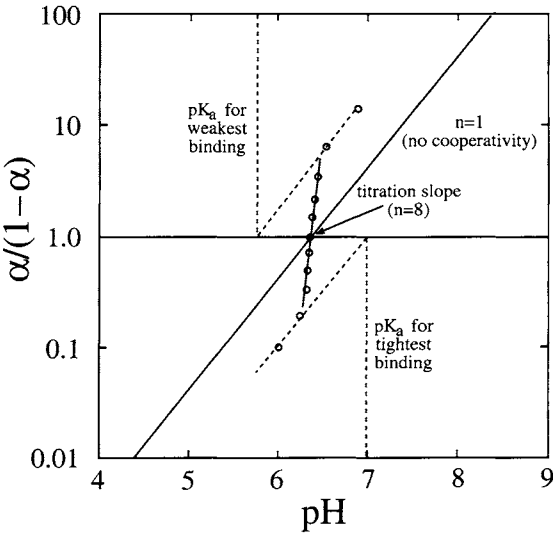

FIGURE 1.4. Hill plot, $\log[\alpha(1-\alpha)]$ vs. pH, for Model protein v: (GVGVP GVGFP GEGFP GVGVP GVGFP GFGFP)$_{42}$. The normal pK for a glutamic acid residue (Glu, E) with E having the ionizable carboxyl group $-COOH \rightarrow -COO^- + H^+$ occurs at a pH of about 4. This plot shows for Model protein v that the first COOH pK occurs at 7.0 and the 42nd COOH pK occurs at about 5.7. Even when completely unfolded and largely disordered, the pK is shifted from 4.0 to 5.7. With the glutamic acid residues separated by 29 uncharged residues in an unfolded, largely disordered model protein, charge–charge repulsion cannot be responsible for the increase in pK. Nonetheless, a repulsion of 2.4 [= (5.7 − 4.0) × 2.3RT)] kcal/mol remains. Noting in Figure 1.2 that the pK shift increases with increases in the oil-like nature of the model protein, the conclusion (along with other data in Ch. 5) is that the pK shift is due to competition for hydration between oil-like and vinegar-like groups constrained to coexist along the amino acid sequence of Model protein v. Of further interest is the change in pK from 7.0 to 5.7 that occurs during the unfolding and dissociation of the oil-like groups within the assembled model protein. During an unfolding fluctuation, oil-like groups become surrounded by special structured water, and, for the first $-COO^-$ to form, the emergent carboxylate must rearrange water around the oil-like groups and organize it for its own hydration. The first carboxylate, having destructured the most water around oil-like groups, makes it easier for the second carboxylate to form, and so on. This is the physical basis for positive cooperativity, which was of particular interest to Monod.

1.2.2.2.2 Two Aspects of the Fully Charged State

The fully charged state lies bare the tense, taut, or cocked state with two aspects. The fundamental aspect is the repulsion between vinegar-like and oil-like constituents along the chain molecule, as reflected in pKa shifts and positive cooperativity. The other aspect is the resulting elastic deformation of chain segments due to repulsion between vinegar-like and oil-like components within the model protein structure. The vinegar-like and oil-like components of the model protein each reach out for water unused by the other. The model protein becomes constrained, even in the more disordered state, as the result of being limited from arrangements where oil-like and vinegar-like constituents would be in closer proximity.

1.2.2.2.3 Analogy Between Fully Charged Carboxylate ($-COO^-$) State of Model Proteins and the ATP^{-4}-bound State of Protein-based Machines

In Figure 1.4, one carboxylate in each 30 residue repeat, (GVGVP GVGFP G*E*GFP GVGVP GVGFP GFGFP)$_{42}$(GVGVP), sustains a repulsion of 2.4 kcal/mol-carboxylate

even when the model protein is completely unfolded. This repulsion arises almost entirely due to the presence of the phenylalanine (Phe, F) residues. Half of the repulsion, that is, 1.2 kcal/mol-carboxylate, remains, even when the two most sequence-proximal F residues are replaced by V. ATP, on the other hand, exhibits four negative charges instead of the single negative charge of a carboxylate. Because of this, ATP produces a much greater repulsion between oil-like and charged groups, even when ion paired with magnesium ion (Mg^{+2}). ATP, therefore, reaches out substantial distances to destroy the structured water around exposed oil-like groups that allows separation of existing ion pairs and to destroy structured water forming around emerging oil-like groups that promotes dissociation of oil-like groups and domains. The central arguments that the development of too much hydration around oil-like groups causes insolubility and that the presence of charge disrupts hydration around oil-like groups to give solubility are developed in Chapter 5 (sections 5.1.3.3, 5.1.7.4, 5.3.3.3, and 5.7.9.2), and illustrated in Chapters 7 (sections 7.2 and 7.5.1.4) and 8.

1.2.3 Assertion 3: The Assertion of Biological Relevance

Biology thrives near a movable transition for insolubilization of oil-like domains, and the forces that, in a positively cooperative manner, power the molecular machines of biology drive-paired oil-like domains of proteins back and forth between association (water insolubility) and dissociation (water solubility); excursions too far in either direction into the realms of insolubility or solubility spell disease and death.

Phenomena exhibited during protein function and dysfunction (disease) parallel those phenomena exhibited during function of designed model proteins. Examples of this coherence of phenomena follow in a cursory introductory form. Detailed considerations are given in Chapters 7 and 8 based on the physical mechanisms developed in Chapter 5.

1.2.3.1 By the Consilient Mechanism Protein-based Machines Require Water to Function

A protein-based machine without water as an integral part of its structure could not function by the consilient mechanism. In other words, water is required in at least one of the two states in order to have a "movable cusp of insolubility," and in order for competition for hydration to be relevant there must be adequate water present. The first prerequisite, therefore, in addressing the biological relevance of the consilient mechanism is to assess whether or not water exists within or between the changing structural elements of a protein motor during function.

1.2.3.1.1 The Myosin Motor Domain of *Dictostelium discoidium*

The myosin motor is an ATPase, because it is driven by cyclic ATP binding to cause detachment, splitting to form ADP and P_i, and the sequential release initially of P_i with contraction and then of ADP in readiness for a new ATP to bind. The organism *Dictostelium discoidium* provides a convenient motor domain for studying muscle contraction because of its near identity to the myosin motor of skeletal muscle.

Protein motors are three-dimensional. Therefore, being able to see in three dimensions is very helpful in order to understand structure and the changes in structure that drive function. A convenient means of visualizing in three dimensions can be obtained by a "stereo view." Two views of the structure of interest are given with one view rotated a few degrees on its vertical axis from the other. Rotation one direction allows for a three-dimensional view when looking with crossed eyes (i.e., as though the image were midway between the printed paper and your eyes), whereas rotation in the opposite direction allows a three-dimensional view when looking wall-eyed (i.e., as if the pair of images were at a distance). Figure 1.5 provides a stereo view of the motor domain arranged for cross-eye viewing.

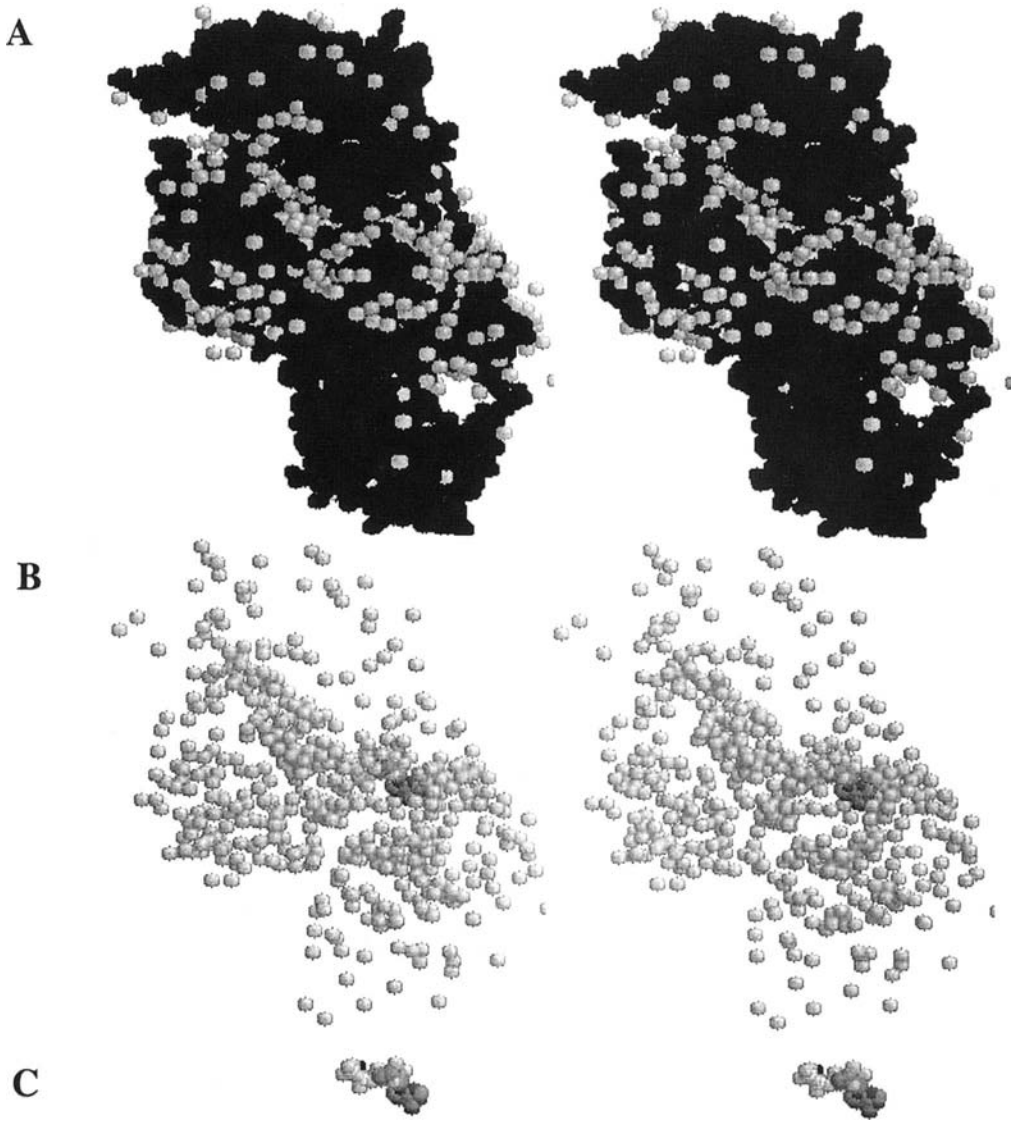

FIGURE 1.5. The "waters of Thales" of the myosin II motor. Stereo views of the crystal structure of the myosin motor domain of *Dictostelium discoidium* in presence of ATP are shown. **A** Space-filling display showing water molecules on a sculptured surface of the myosin II motor. **B** Those water molecules throughout the entire structure that are sufficiently fixed in space to be seen by X-ray diffraction are shown by removal of the protein component. These waters and additional water in the surface crevices are the "waters of Thales" required for function by the consilient mechanism. **C** The ATP is shown in the same orientation with all other atoms hidden. (Figure preparation was based on the crystallographic results of Bauer et al.[11] as obtained from the Protein Data Bank, Structure File 1FMW.)

Our special interest at this point is to gain insight into the presence and distribution of water within and around a motor domain, as would be required for the consilient mechanism to be relevant to motor function. Because it shows a recently determined structure,[11] Figure 1.5 represents an up-to-date capacity for locating water molecules. This structure of the myosin motor contains ATP, as shown in Figure 1.5C, with all other atoms hidden.

1.2.3.1.2 The Sculpted Appearance of the Myosin Motor Domain

Especially when seen in three dimensions, as in Figure 1.5A, the stereo view of the myosin motor domain has the appearance of a sculpted surface. The surface contains crevices and depressions, as though formed from sandstone that had been weathered by wind and rain. Only a relatively few water molecules are seen in these surface recesses, because the majority of water molecules are too mobile to be observed by X-ray diffraction. Yet these surface crevices and depressions can be filled with water molecules that, by the consilient mechanism, contribute to the energy considerations of motor function. In this regard, it should be appreciated that only 10% to 20% of the existing water molecules are sufficiently fixed in space to be located by X-ray diffraction.[12]

1.2.3.1.3 The "Waters of Thales"

When the space-filling protein component is removed, it becomes possible to view the located water molecules within the myosin motor. As shown in Figure 1.5B, an impressive number and distribution of the detected water molecules appear. It can also be expected that there are many more water molecules relevant to function of the myosin motor that are too mobile to be seen by X-ray diffraction, just as is apparent in the crevices and recesses of the surface. By the consilient mechanism these water molecules (seen in Fig. 1.5B and the additional unseen water molecules) are essential to motor function. These water molecules, which in our view are essential for Life, we choose to call the "waters of Thales."[13,14] Thus, as required for this protein motor to function by the consilient mechanism, internal water molecules do exist. Accordingly, in our view, this fundamental protein motor that produces motion contains ample water as part of the structure in order to function in the competition for water between oil-like and vinegar-like groups, which competition expresses as a repulsion between these groups.

1.2.3.2 ATPase, Biology's Workhorse Protein-based Machine

In general, ATP (adenosine triphosphate or an equivalent nucleoside triphosphate, NTP) powers Life's protein-based machines. Specifically, the breakdown of ATP to form ADP (adenosine diphosphate) and P_i (inorganic phosphate, PO_4^{-3}) provides the energy that powers protein-based machines. As will be argued in Chapter 8, section 8.1.11.2, the large amount of energy released on ATP breakdown results from the limited availability of water to ATP. Also, by the consilient mechanism, if a pair of oil-like surfaces forms too much special oil-like hydration during a transient separation, they reassociate. Should ATP bind with its multiply charged and thirsty triphosphate tail directed toward the pair of dissociable oil-like surfaces during transient separation, the triphosphate tail recruits the water adjacent to the oil-like surfaces for its own hydration; too much oil-like hydration no longer forms, and the pair of oil-like surfaces remain dissociated. This is the essence of the consilient mechanism; charged (vinegar-like) groups compete with oil-like groups for hydration. Therefore, we ask, might the consilient mechanism dictate a common structural motif for ATP binding to ATPases?

In fact, the ATPase of skeletal muscle exhibits a commonly recognizable structural feature for the ATP bound state.[15] So in an initial illustration (Fig. 1.6), we approach ATPases with the most simplistic cycle, that of an "idling" motor. Then, in Chapter 2 we take a first step toward useful function by attachment to and detachment from a surface and progress from there to more complete depictions. Once the basic science is laid out in Chapter 5, a detailed molecular description is given in Chapter 8.

Like a car in neutral with its motor running, an idling motor runs and consumes energy without producing useful motion. Figure 1.6 depicts an "idling" ATPase motor by means of a cross section of a globular protein that contains the ATP binding site. ATP binding opens a cleft, a cleft that had been closed, or partially so, by association of paired oil-like domains.

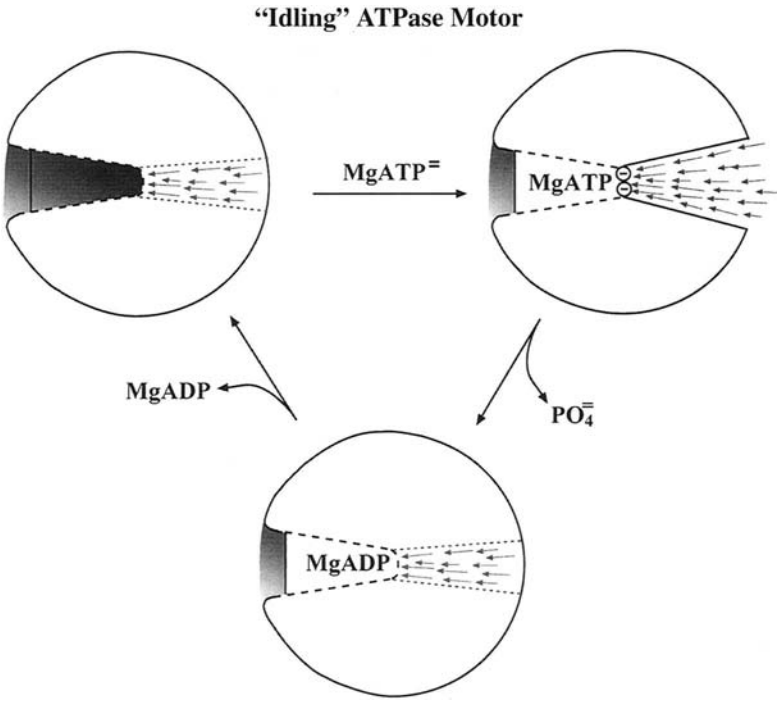

FIGURE 1.6. The "idling" ATPase motor consumes the energy released on conversion of MgATP to MgADP and P_i without the performance of useful work. On binding MgATP to the globular apoprotein, a cleft, partially or entirely closed by association of oil-like groups, opens near the base of the nucleotide triphosphate (NTP) binding site, with the super vinegar-like triphosphate tail at the interface. Release of phosphate allows some recovery of significant oil-like association, and, on release of MgADP, the apoprotein is recovered to complete the cycle.

The ATP molecule orients with its charged triphosphate tail at the base of the cleft such that it can access the water of the cleft. Because of this, water, which would normally form around the oil-like groups and effect reclosure, becomes oriented toward the phosphates and maintains the cleft in the open state. On hydrolysis of ATP and phosphate removal, the cleft partially or completely closes. Release of the ADP molecule recovers the original state to complete the cycle. Accordingly, energy has been consumed, but in this "idling" ATPase motor no work is accomplished.

1.2.3.3 Muscle Contraction

Again, consider statements grouped as bulleted lists with each list defined by a heading.

1.2.3.3.1 Structural Description of the Sliding Filament Model of Muscle Contraction

- Overall in muscle contraction, calcium-ion-binding triggers release of phosphate (the paramount vinegar-like group) with the consequence of contraction.
- The shortening of contraction results from thick filaments sliding past thin filaments.
- A cross-bridge from the thick filament causes the movement by cyclic attachment to and detachment from the thin filament.
- Specifically, the cross-bridge detaches from the thin filament, reaches forward, reattaches to the thin filament and contracts sliding the thin filament past the thick filament.

1.2.3.3.2 Muscle Contraction as Inferred from Model Protein Studies

- Stepwise, ATP binding to the cross-bridge causes detachment from the thin filament by bringing about dissociation of oil-like domains.
- Binding of ATP also causes separation of oil-like domains within the cross-bridge in a way that allows forward extension of the cross-bridge.
- Positive calcium ions pair with negative vinegar-like groups associated with the thin filament to uncover an oil-like domain for a new cross-bridge attachment site.
- Phosphate release reestablishes oil-like association between cross-bridge and thin filament and the power stroke occurs on re-establishment of oil-like associations within the cross-bridge.

This perspective, developed on applying the elastic model protein studies to crystal structures of scallop muscle, brings us to the stage longingly sought by Perutz in 1990 with the statement "that one day we shall learn how chemical energy is generated and turned into motion."[16] Interestingly, at the time Perutz penned these words, the demonstration of chemical energy turned into motion by the unexpected source of designed elastic-contractile model proteins of primary focus here had been in the literature for little more than one year.[17] Figure 1.7 gives the key results of that publication in part A for acid-driven contraction at constant force (isotonic contraction) and in part B for acid-driven force development at constant length (isometric contraction).[17] The fundamental process, regardless of the experimental conditions, is acid-driven association of oil-like domains.[18]

The movable cusp of insolubility represented in Figure 1.1 provides a means of visualizing the molecular process. Addition of acid (proton, H^+) converts the vinegar-like carboxylate group

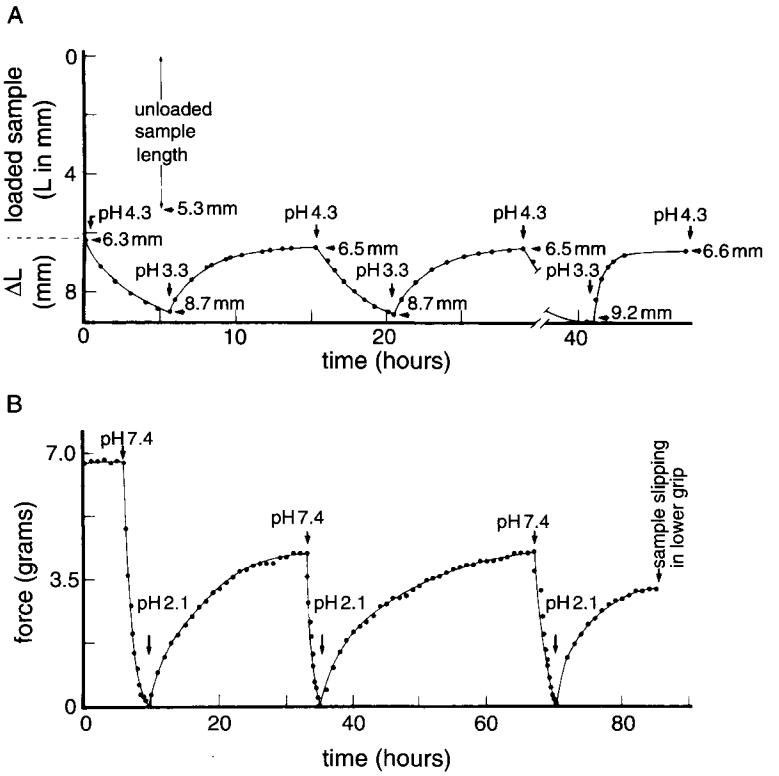

FIGURE 1.7. Shown are the first reported data[17] of the conversion by an elastic-contractile model protein of chemical energy due to an increase in concentration of acid into the mechanical work of contraction. **A** Length changes at constant force (isotonic contraction) in phosphate-buffered saline. **B** Force changes at constant length (isometric contraction) in phosphate-buffered saline. (Reproduced from Urry et al.[17])