OPEN ACCESS

BIO-complexity

Research Article

An Engineering Perspective on the Bacterial Flagellum: Part 1—Constructive View

Waldean A Schulz*

Conceptual Assets, Spokane, Washington, USA

Abstract

This study examines the bacterial flagellum from an engineering viewpoint. This examination concentrates on the structure, proteins, control, and assembly of a typical flagellum, which is the organelle imparting motility to common bacteria. Two very different, independent approaches are applied and then compared in three separate papers: Parts 1, 2, and 3. The first approach is a *constructive* or *top-down* approach, covered in this Part 1. It considers the purpose of a bacterial motility system, its typical environment, new and existing required resources, and its physiology. It sets forth the logically necessary functional requirements, constraints, assembly, and relationships. The functionality includes a motility control subsystem and provision for self-assembly. The specification of these requirements is intended to be independent from knowledge of the flagellar structures. This is original material not covered in academic papers on the flagellum. Part 2 will cover the second approach, an *analytical* or *bottom-up approach*. It will document the known 40+ protein components and the structure, assembly, and control of a typical flagellum. The bacterial flagellum is a well-researched molecular subsystem. However, in Part 2 the assembly relationships will be illustrated graphically in a form and detail not found in previous literature. Part 3 will compare the two approaches and conclude with several original observations. Those include the coherent assembly orchestration and an ontology of the exceedingly specific protein-binding properties. The latter observation is significant, and it suggests future modeling to elucidate how the strong, coherent, multi-way protein binding is achieved at the molecular level.

Cite as: Schulz W (2021) An Engineering Perspective on the Bacterial Flagellum: Part 1—Constructive View. BIO-Complexity 2021 (1):1–14. **doi:**10.5048/BIO-C.2021.1.

Editor: Robert J. Marks II

Received: December 4, 2020; **Accepted:** March 27, 2021; **Published:** July 2, 2021

Notes: A *Critique* of this paper, when available, will be assigned **doi:**10.5048/BIO-C.2021.1.c.

*dean@conceptual-assets.com

INTRODUCTION

Systems biology [1] employs methodology and techniques typical of systems engineering. Similarly, reverse engineering the features of biological organisms leverages both biology and engineering disciplines. Specifically, the systems engineering perspective on bacterial motility detailed in Parts 1, 2 and 3 studies the purpose, functions, components, and structure of a typical bacterial flagellum and the flagellum's assembly stages. The dynamic operation and control of this motility organelle is also studied. This study takes two essentially independent approaches below. One is a constructive approach, which this Part 1 covers; the other is an analytical approach to be covered in Part 2.

The first, *constructive approach* is a *top-down specification*. It starts with specifying the purpose of a bacterial motility organelle, the environment of a bacterium, its existing resources, its existing constitution, and its physical limits, all within the relevant aspects of physics and molecular chemistry. From that, the constructive approach derives the logically necessary functional requirements, the constraints, the assembly needs, and the hierarchical relationships within the functionality. The functionality must include a control subsystem, which needs to properly direct the operation of a propulsion subsystem. Those functional requirements and constraints then suggest a few—and only a few—viable implementation schemata for a bacterial propulsion system. The entailed details of one configuration schema are then set forth.

This constructive approach is analogous to how a myriad of theorems, definitions, and constructions of plane geometry are derived from the few basic axioms and the rules of logic. A sincere attempt has been made to keep the elaboration of this

constructive approach logical and as independent as possible from knowledge of the actual flagellar structure.

The *analytic approach* of this study will be covered in Part 2 and is a *bottom-up* deconstruction of a typical flagellum. The bacterial flagellum is a well-researched molecular subsystem, and Part 2 draws its information from many cited papers. It documents the known 40+ protein components and the observed and inferred structure, assembly, and control of a typical flagellum. However, in Part 2 the protein and assembly relationships will be illustrated graphically in a form and detail not found in any previous paper.

After the constructive and analytic approaches are presented, they will be compared in Part 3 along with a set of fresh concluding observations. The comparison is appropriate, because engineers regularly specify and design systems top-down, but they construct those systems bottom-up. Then the resulting implemented system is evaluated against the specification.

A REVIEW OF ENGINEERING METHODOLOGY

A common engineering methodology, called the Waterfall Model [2][3], first produces a formal Functional Requirements Specification document [4][5][6][7][8]. Then a design is proposed in a System Design Specification, which must comport with the Requirements Spec. Typically this methodology is often accompanied by a Testing Specification, which measures how well the subsequently constructed system satisfies the requirements. This methodology was and is successfully applied at Intel, Image Guided Technologies, and Stryker. A similar specification method can be used by a patent agent or attorney in helping inventors clarify in detail what they have invented for a patent application.

The methodology begins with a statement of the overall purpose for the proposed system, the usage environment, necessary functionality, available materials, tools needed for construction, and various parameters and constraints (dimensions, form, cost, materials, energy needs, timing, costs, and other conditions). Included in the constraints are the manufacturing and operational needs (energy, tools, assembly jigs and templates, protocols or standards, resources and materials, scheduling, supply chains, documentation, and distribution chains). Then a design is proposed that logically comports with those requirements. The design specification may include viable implementation options, subsystem hierarchy, and production assembly to provide the required functionality, the purposes of the individual parts, implied attributes, necessary structural or logical interrelationships, costs, and the rationale for each detail.

While there may be alternative designs, the requirements strictly limit the number of viable solutions to a very few alternative designs, each of which must still manifest all required functionality and constraints. Any alternative viable designs likely will exhibit different characteristics and trade-offs with respect to various non-functional requirements, such as energy efficiency, speed of operation, elegance, methods of assembly, and so forth.

Such a top-down methodology is commonly used for specifying the requirements and design for bridges, medical devices, software, and a wide variety of engineered products and projects—frequently employing a combination of cross-disciplinary engineering, physics, chemistry, and mathematics.

REQUIREMENTS FOR BACTERIAL MOTILITY

The following applies a similar engineering approach to a motility system for bacteria. In doing this, the constructive approach becomes—in effect—the engineering documentation that must be written as if a clever bioengineer were tasked to devise a motility system for a bacterium lacking a motive organelle. This is done to follow a typical systems engineering approach, which produces a detailed design from functional requirements, but it does not presuppose the existence of any such intelligent agent. However, this approach can be an instructive exercise in determining how many logical requirements are actually dictated by the need for a motility organelle for a bacterium. In effect, this top-down specification is intended to be an independent, detailed "prediction" of a motility system for bacteria, albeit a prediction after the fact.

This approach starts with the purpose, context, and obviously necessary functionality—presumably excluding knowledge of an actual biological implementation. Although this approach surely can be accused of being unconsciously guided by the known biological realization (a flagellum and its chemotaxis), the earnest attempt by the author has been to independently derive the necessary structure and subassemblies from the primary purpose and context. There is one exception noted below, which is used solely to avoid unnecessary effort.

What would be the context, the generic functions, and the constraints for a motility system for a bacterium lacking motility? Which materials, functionality, structures, assembly, sensors, signaling means, and operational control are required? How would such a motility system be appropriately divided into subsystems and into subassemblies, each with its own individual purpose and functionality, which coherently contribute to the whole purpose and overall functionality? Such questions are the basis for refining the logically required configuration of a complex subsystem.

To aid in separating the logical top-down functional requirements from the known details of actual flagella, the following specification will mostly avoid using the usual microbiological terms—instead employing more generic, engineering terminology.

Subsystem Context and Functionality

The environment of a typical bacterium generally may include both nutrients and deleterious substances. Further, the bacterium typically is suspended within a liquid or semi-fluid medium. This suggests a constraint that a "leg-like" means requiring motility for a bacterium would not be efficacious, because the bacterium is suspended (unlike a crab or lobster, for which gravity and its mass provides traction on a firm bottom surface).

The overall function of the bacterial motility system accomplishes two purposes. First, the system should enable a

bacterium to sense and move toward nutrients needed for metabolic energy, self-repair, and reproduction. Second, the system should enable its bacterium to sense and escape hostile locales, such as toxic or noxious material.

Therefore, a motility system implies a *propulsion subsystem* to accomplish motion. Searching for nutrients or for an escape route further requires some form of primitive *redirection subsystem*, working in concert with or integrated with the propulsion subsystem.

Further, a collateral *control subsystem* must exist to sense favorable or unfavorable substances—preferably both, to make logical choices, and to appropriately control the propulsion and redirection subsystems through some signaling means. Otherwise, the propulsion and redirection subsystems are a waste and cannot achieve the overall purposes. So, the control subsystem comprises three subassemblies: sensors, decision logic, and output signals to affect the operation of the propulsion and redirection subsystems.

The purpose requires that the propulsion subsystem must provide at least two states in response to those control subsystem signals: forward motion and stopping/reverse. Simply stopping all motion accomplishes little, since Brownian motion would eventually jostle the bacterium into a new, random orientation. Further, stopping would conserve energy expenditure particularly *but only if* nutrients were immediately available. Yet simply providing reverse propulsion would only provide backtracking and not provide a means to search new, more favorable environmental locales. A more direct means to change direction is needed during stopping or reversing. Because the sensors only work on intimate contact (not at a distance), and because the bacterium is so small, it would not be locally obvious which new direction would be more advantageous. Determining the most favorable reorientation would require considerably more complexity: more sensors, more intricate control logic, a more elaborate redirection subsystem. That surely would expend more resources of the bacterium: energy, new custom material, more involved assembly. Therefore, rather than some elaborate "steering" means, a simple *randomized* redirection not only suffices but is a better solution than simply always turning in the same direction. Repeatedly turning the same direction would lead to a circular path. The means to change direction may be implemented as a separate redirection subsystem (like the rudders on a submarine) or be integrated as a part of the propulsion subsystem proper (like the gimballed combustion chamber of a rocket engine).

The sensors need to provide sensory input to the control system's decision logic, which quantitatively compares the amount of the desirable or harmful substance with thresholds. The thresholds may be fixed but would be more effective if there were short-term memory of input. If so, the increasing or decreasing gradients of substance concentrations could then be detected. Further, the control subsystem needs to connect to the propulsion and redirection subsystems through control signals, which would include two signals: "full speed ahead" or "flee and redirect." Either signal might be the default, so that absence of the other signal is in effect an implicit control signal.

One specification question is this: How many sensor assemblies do there need to be? Do there need to be more than one for redundancy? Is redundancy required? If more than one sensor assembly is present, how are multiple inputs evaluated? Voting scheme? Summation of some sort? Does one sensor's flee-and-redirect signal veto several other sensors' full-speed-ahead signals? What is the assembly and operational cost of multiple sensor assemblies? To keep the motility system as simple as possible, and because one sensor assembly seems necessary but sufficient, a minimum of one sensor assembly is specified herein.

Often a system's specification contains a list of optional features. These would be other desirable functions, included in the specified system because secondary purposes may suggest them, and they may be included as resources allow. For example, there could be more than a single propulsion subsystem per bacterium, if multiple propulsion subsystems would be efficacious. In that case, there surely would be logically necessary constraints on their location, arrangement, and coordination of propulsion direction and redirection. However, the following discussion will ignore those options and generally focus on the case of a single propulsion subsystem and redirection subsystem, along with a companion sensor and control subsystem.

Another example of an optional feature would be the ability of the bacterium to dismantle or eject the propulsion subsystem under dire circumstances. Further, there could be some means for multiple bacteria, each equipped with the propulsion system, to cooperate. But again, such options will not be considered for the specification, because the goal is to specify only a *minimal* set of requirements, assuring that all the requirements of the subsystem are essential. That would imply that the specified sensory-propulsion-redirection system is *effectively irreducible*. That is, if some part is missing or defective, then, at best, there would be noticeably diminished motility, if any.

Operational Constraints

The coherent control, propulsion, and redirection subsystems must be agile enough to sustain the life of its bacterium. First, the response time between sensing a nutrient or a threat and changing forward motion and/or direction must likely be less than a few milliseconds because of the diffusion rate of beneficial or harmful substances at this microscopic scale.

Second, the propulsion speed needs to be sufficiently fast, especially for the purpose of escaping threats. It seems that one body diameter (about 1 to 10 μm) per second (to an approximate order of magnitude) would be a requisite minimum velocity in its fluid environment. A substantially faster speed would be wasteful of energy and perhaps dictate a more complex design. At the bacterial scale of a few microns, Brownian motion is a dominating force; inertia is not. We can no longer consider water a continuous fluid but instead a composition of molecular particles. These are characterized by stochastically distributed collisions at life-sustaining temperatures, so viscosity becomes more obviously related to the Brownian motion at this scale.

This specification includes some estimated reasonable

response and speed values, but a biologist familiar with bacteria might suggest better values. Regardless, the proposed values are indicative of very real physical-chemical constraints related to survivability, and the values must be neither too large nor too small. Obviously a very hazardous environment would suggest a faster response and escape speed, so there is a trade-off optimizing the response against other factors in a more typical environment.

The energy cost to operate the propulsion subsystem must be less than the energy obtained by navigating to and consuming nutrients. Otherwise, the bacterium would soon die of starvation. This involves energy quantities with real limits. For the above minimum speed of, say, 1 μm/s, the required force to move a bacterium is about 10^{-4} dyne, or 10^{-9} N [9]. Therefore, the energy required to move one body length is at least 10^{-15} J. The power to do that in 1 s is 10^{-15} W.

Assembly Constraints

The material resources and energy requirements to build a propulsion system must be low enough to justify its construction—that is, to justify the benefit of motion to find new nutrients for metabolism. Again, this involves energy quantities with real-world limits.

The assembly time to build a flagellum would preferably be less than the time for cell division. This is not a strict requirement, but it seems that cell division could interfere significantly with the flagellar assembly process, such as separating nascent sensor and propulsion subsystems.[1]

A very important assembly constraint is that the various logical components of the flagellum (discussed below) are assembled in an orchestrated order of assembly.

The environment is assumed to be liquid or semi-fluid (perhaps very viscous) and so implies some constraints on the structure of a propulsion subsystem. Any such subsystem design will undoubtedly require some external mechanical means of interacting with the surrounding environment in a way that produces motility: an appendage, paddles, wheels, propeller, or other structure.

Any such motility schema presents assembly challenges. Assembling any protruding appendage *outside* the cell would expose the nascent appendage to many potential hazards and would require some kind of external scaffolding and protection from the potentially hostile external environment. If, instead, the external appendage is assembled *inside* the cell, there would be the trade-off as to whether the growth should occur at the distal or proximal end or all along the length of the appendage. Growth at the proximal end would require that the nascent appendage remain attached as new constituents of the appendage are inserted between the main cell body and the existing partial appendage. That would necessitate specific considerations to keep the appendage attached during assembly. Alternatively, growth at the distal end or along the length of the appendage would require having an internal delivery path and conveyance for its constituents during assembly.

In any case, the assembly process may require piercing the cell wall somewhere, but since leakage into or out of the bacterium would be catastrophic, this must be done in such as way as to preserve the cell wall's integrity. This is yet another essential specification constraint.

The specification of the *assembly* of a substantive coherent system is generally at least as complex as the final *structure* of the system. In the commercial world, think of building a skyscraper too tall for external cranes, so that components need to be designed to be delivered through an elevator shaft; or think of mail-order furniture which requires assembly by the buyer, requiring instructions, specific assembly order, bolts, panels, legs, supplied wrenches, and their organization—all likely to differ from how the furniture would be assembled within a factory. In the case of a bacterium, all assembly must occur while the bacterium maintains normal metabolism. Further, propulsion subsystem assembly and operation must not interfere with reproduction and vice versa.

A DESIGN FOR BACTERIAL MOTILITY

Thus far we have discussed the *functional* requirements and the logically related constraints and implications—parallel to what a practicing engineer would expect to see in a Functional Requirements Specification for a proposed real-world system. Now we turn to an implied system design that would meet the Requirements Specification, a design which a practicing engineer would express in a System Design Specification. It will begin with the design for any system that would meet the functional requirements, then turn to the limited choices for a system design schema and its logically necessary components.

Subsystem Subassemblies

What then are the logical subassemblies, which surely must be present to produce a viable combination of motility subsystems, which fulfill the ultimate purpose as well as the logically implied functional requirements?

First, the propulsion subsystem needs a source of power to operate. Most of a bacterium's operational energy is supplied by the ubiquitous ATP-to-ADP or ATP-to-AMP conversion. This seems a likely candidate for propelling the bacterium. However, there is at least one other option for harnessing power: the electrical ionic (pH) differential maintained between the cytoplasm and the peptidoglycan layer outside the inner (cytoplasmic) membrane. This is the very means of power used to produce ATP.

Second, there must be a power-to-motion transducer. It must convert the power into motion. The choice of the source of power and the implementation of the transducer are intimately correlated.

Next, there must be sensors to detect whether the propulsion system should move the bacterium forward. Detecting "whether" requires decision logic. The logic could be a simple comparison of the level of the sensors' activity to a fixed threshold. However, to be as effective as, say, a thermostat on a home furnace, there should be some built-in *hysteresis*, a

[1] One could, however, imagine a cell dividing into a daughter cell, which has not yet begun construction of flagellum, while the "mother" cell possibly has completed or is still in the midst of flagellar assembly.

simplistic short-term history in effect. Hysteresis is common in human-engineered control systems such as the thermostat. Hysteresis prevents sudden, wild swings during operation and would enable detection of positive or negative gradients within nutrient or noxious environments. The decision logic in turn must communicate the result of its evaluation to the propulsion system via a control signal, possibly implemented as an expressed chemical that actually affects motility. Other means of signaling (mechanical or electrical, for example) can be imagined but seem too complex or foreign to the usual capabilities of a bacterium. These three necessary subassemblies form an integrated control subsystem, which may be centralized or distributed. The propulsion system must respond appropriately to the control signals, both functionally and within a suitably quick time.

Multiple propulsion subsystems could provide a more elaborate redirection system. But providing differential control signals to them would potentially require more control logic, as well as multiple sensors in cell wall locations coordinated with the locations of the multiple propulsion systems. This goes beyond the stated goal of a minimal specification.

As noted above, there must be some external member physically interacting with the environmental medium containing the bacterium. The appendage must be moved by the energy-to-motion transducer. This suggests some provision to ensure that the motion of the appendage does not expose the cell's inside to its environment. Therefore, some kind of seal or flexible gasket is required between the cell membrane(s) and the external appendage.

ASSEMBLY

Before the propulsion subsystem can fulfill its purpose, the subsystem must be assembled. That includes all the aspects, steps, and constraints discussed in the following sections.

Construction materials

While the putative clever bioengineer of the proposed propulsion subsystem could theoretically choose materials other than proteins, what would those be? Sugars? DNA/RNA bases? Because there already exists all the exquisite cellular machinery supporting protein fabrication—genes with binding and termination sites, sigma factors, mRNA/tRNA, ribosomes, and so on—the obvious choice of materials would be proteins. The next question is whether new proteins are required beyond preexisting cellular proteins. Because the propulsion system adds new functionality and will require components with special functions (each uniquely contributing to the whole purpose of motility), at least some novel, customized proteins will undoubtedly be required even if some existing polypeptides can be repurposed. For example, the new functionality includes specialized chemical sensing and orchestration of the order of assembly.

There are already all the available "fabrication tools" for the materials and parts, such as RNA polymerases and ribosomes. There must be new DNA "blueprints" for fabricating the novel proteins. Control of protein fabrication generally involves co-factors, promoters, chaperones, and the like. Thus, an obvious generic requirement of using available fabrication tools, templates, and control effectively rules out the use of other materials, such as sugars or non-protein polymers.

Tools and Templates

As just mentioned, there are required, and also available, tools for fabrication of proteins. But novel proteins will require novel templates—genes—and activation of those templates needs to be coordinated with specific steps of assembly (to be specified below).

Further, for assembly proper, it will surely require some temporary scaffolding or active, specialized construction tools (presumably also proteins) to sequence, place, and align the structural proteins of the propulsion subsystem (and redirection and control subsystem).

Any propulsion system satisfying the above functional requirements will face some common assembly challenges (as are faced by assembly of any other organelle). First, there must be some delivery means to move all required proteins to the assembly site from the ribosome that fabricated them. The delivery means could be simple diffusion, but that would slow the delivery of a specific protein to the right location. Producing a plethora of copies of the protein would speed the process, but that would burden the bacterium with unneeded work and would interfere with delivering the right proteins to the right spot in the right sequence. A more direct delivery supply chain would speed assembly, but would require design of that mechanism, unless such a subsystem already existed.

Second, there must be some way to ensure that the proteins are properly oriented and inserted into the correct position. Once a protein is near its destination, attractive electrostatic forces of a very specifically designed protein could provide the orientation and placement means.

Third, there must be control over the order in which the component proteins are delivered and inserted.

SPECIFIC SYSTEM DESIGN CANDIDATES

The above sections have described the generic subsystems and their subassemblies logically required by the functionality requirements for a propulsion system. This section will consider the alternative schemata for the power-to-motion function but will focus on one schema. There really are only very limited generic candidates for viable mechanical power-to-motion schemata for bacteria within a fluid or semi-fluid medium.

One candidate schema could use *squirting* to implement conversion of power to motion. For example, it could employ a fluid-filled bladder with an output "nozzle" to propel the bacterium forward—much like a rocket. That would necessitate a further means to refill the bladder and some means to control the direction of the squirted fluid to achieve the required redirection function. If the fluid were provided from within the bacterium, that could quickly deplete the bacterium of its internal cytosol. If the bladder filled from one end of the bacterium and squirted out the other end, valves of some form would be necessitated to open and close when the bladder

refilled and contracted to squirt its contents. If the fluid were simply sucked back into the bladder, the bacterium might then simply backtrack to its starting location with no net gain in forward position—unless the bladder and nozzle appropriately changed shape between filling and squirting or the filling was done slowly and the squirting vigorously.

A second candidate schema is a rhythmic *flexing* of the whole body. The jellyfish uses a coordinated contraction of its umbrella-like body to produce a vortex. That is followed by a recovery stroke, so configured that it does not simply pull the jellyfish back to the location it occupied before the contraction. A human swimmer doing a breaststroke illustrates this also. A variant of this is a rhythmic *wiggling* schema. It is used by sperm, fish, and human swimmers doing a dolphin stroke. (The latter works underwater, unlike the crawl or freestyle stroke, which is ineffective for a fully submerged swimmer.) Such a propulsion means would require a flexible cell wall and some power-to-motion transducer to flex or wiggle the cell's shape. In addition, this design—like all others—would require a power source, a sensor subsystem, communication between the component subsystems, decision control with feedback, and so on.

A third specific candidate schema might use *leg-like appendages*. Something like oars would not work well when fully immersed in a semi-liquid environment. Something like a swimmer's arms in the breaststroke is more like the flexing schema above. True leg-like appendages, requiring contact with a more solid surface (as in the motion of a starfish or crab), would fail when the bacterium was suspended within a liquid or semi-liquid environment above a surface. At best, legs would poorly fulfill the propulsion requirement.

A fourth specific candidate schema could use a snake-like or caterpillar-like crawl. As with legs, it would require contact with a solid or semi-solid surface in the environment. Again, it is a poor candidate for propulsion for full submersion in a liquid and short of the functional requirements specification. For similar reasons and others, wheels would not be a candidate schema.

All the above candidate schemata have advantages, disadvantages, and trade-offs. All would require at least one contracting and relaxing muscle-like organelle as the energy-to-motion transducer of the general requirements. Each schema, of course, would logically imply a whole set of further design details.

A fifth specific candidate schema would use an external, rotating, *helical propeller*. Note that an external wheel, like a paddle wheel steamboat, would not work for a completely submerged bacterium. This is why a helical geometry is necessitated. This schema will require a rotary energy-to-motion transducer—a motor—as well as an energy source, and sensors as in the above schemata.

Perhaps further, alternative schemata are conceivable for inducing motion for a bacterium, but the above schemata are the obvious ones and are exemplified in other biological organisms or in mechanical devices made by human designers.

While it could be instructive to elaborate the further logically necessitated design details for a wiggling propulsion system or any of the other schemata above, this is not the place for that. Instead, we will elaborate only the necessitated design details for the rotary schema. Of course, that is exactly what a real-world bacterium has, so pursuing just this one schema is an exception to the goal of independence from knowledge of the bacterium flagellum. However, (a) any of the alternative candidate schemata would surely have a detailed intricacy comparable to that of the rotary schema, if we were to particularize those schemata; and (b) the following logically implied details of a rotary propeller design will be derived independent of what is otherwise known about the flagellum's details.

Rotary Design: Implied Details

The energy source for a rotary design ideally would supply continuous power, or nearly so. Two power sources would readily be available. One is the ubiquitous ATP-to-ADP or ATP-to-AMP energy units. The other is to directly use the electrical potential existing across the cytoplasm membrane, which is due to an ion or pH differential, itself used to power production of ATP.

To generate the required torque, there will need to be rotary and static subassemblies. The rotary subassemblies would provide rotational torque. The static subassemblies would provide stability and provide counter-torque from a rigid part of the cell—presumably the outer cell wall, peptidoglycan, and/or the plasma membrane.

The rotary schema must have the following rotary subassemblies: an *armature* or mounting structure, a *motor rotor*, a *drive shaft* of appropriate length, a helical propeller, and possibly *adaptors* to bind those components together. In some cases (see below) there may need to be a *torque axis redirection means* between the drive shaft and the propeller.

The rotor may act as the armature, but there are two different functions: the rotor is needed to produce torque in concert with the stator (see below); the armature is needed to connect the rotor to the drive shaft and to aid the assembly of the rod and propeller. The drive shaft is needed to transmit the torque to the exterior of the cell, traversing the peptidoglycan and the outer cell membrane. During assembly, the rod must be capable of penetrating them. The helical propeller is the necessary rotary-to-forward-motion transducer. The propeller could be multi-bladed, as is typically used on ships, or could have a corkscrew geometry, but the latter has the advantage of less drag (at least at macroscales) and may have advantages with regard to Brownian motion. In any case, the propeller must be relatively rigid and robust.

Further, there need to be bearings and seals between the rotary components and static components. Can the seals wear or is there van der Waals "stiction" between the rotary and static components? There surely are further and unique challenges at the molecular level not apparent at the macro-level "mechanical" scale.

In certain cases, there may need to be an additional means for redirecting the torque axis rearward. If a single propulsion system is mounted to the polar end of the bacterium, for example, this torque-axis-redirection-means is not required. But if there were to be multiple propulsion systems, which cannot all be

collocated at the polar end, they must be located at lateral locations on the cell, and so require their torque axes to be directed to the same direction (presumably rearward)—perhaps bending each system's torque axis by as much as 90 degrees. A typical mechanical U-joint would not work because U-joints lock up at 90-degree bends. A flexible shaft, which is still torsionally rigid, would satisfy the requirement.

The static subassembly requires the following components: the semi-rigid cell membrane(s) for rigid mounting, a *motor stator*, multiple sealed bearings where the rotary subassembly penetrates cell membranes, and an energy conduction pathway.

The stator together with the motor rotor produces torque. The stator must be rigidly attached to some or all of the bacterium's inner and outer membranes and the peptidoglycan layer. The rigid attachment transfers necessary counter-torque to the cell body as well as providing stability for the rotary subassembly. For each membrane or layer the drive shaft penetrates, there must be a bearing. Each bearing must (a) stabilize the drive shaft, (b) provide a low-friction contact with the drive shaft, and (c) provide a seal to prevent movement of molecules past where the shaft penetrates its host membrane or layer.

To construct the components extending outside the cytoplasm there must be some kind of conduit for the constituent structural proteins and an injector for sequencing and directing proteins into the conduit. While it may not be logically required, it seems that the conduit for simplicity should be inside the drive shaft and an injector must be attached to the rotary subsystem—presumably attached to the armature. That is because the conduit and injector must be directly involved in the construction of the rotary subsystem's shaft and propeller (and also the torque-axis-redirection means for multiple off-axis propulsion subsystems).

A REQUIREMENTS AND DESIGN DEPENDENCY NETWORK

A graphical network (also called a dependency graph) can better depict the above requirements and their interdependency relationships. The relationships are represented as edges between two nodes, where each node represents a specific requirement detail. A requirements network for specifying a propulsion subsystem for a bacterium is shown in Figures 1 through 4. The network formally captures the specification details discussed above: purpose, environment, required functions, constraints, and the logically implied static, structural requirements.

Figure 1 is the legend for the meanings of the 18 types of edges appearing in the following three figures. Because the whole network is too large to be legible on one page, the network is split into three subnetworks: Figures 2, 3, and 4. The labeled nodes in the dashed boxes in one figure are the nodes with the same label in another figure. For example, "power to motion schema" in Figure 2 appears as the root node of the subnetwork in Figure 3.

Certainly, hundreds of variant specifications for a bacterial motile system (and networks describing them) are possible. Many would provide even further elaboration. Nevertheless, all

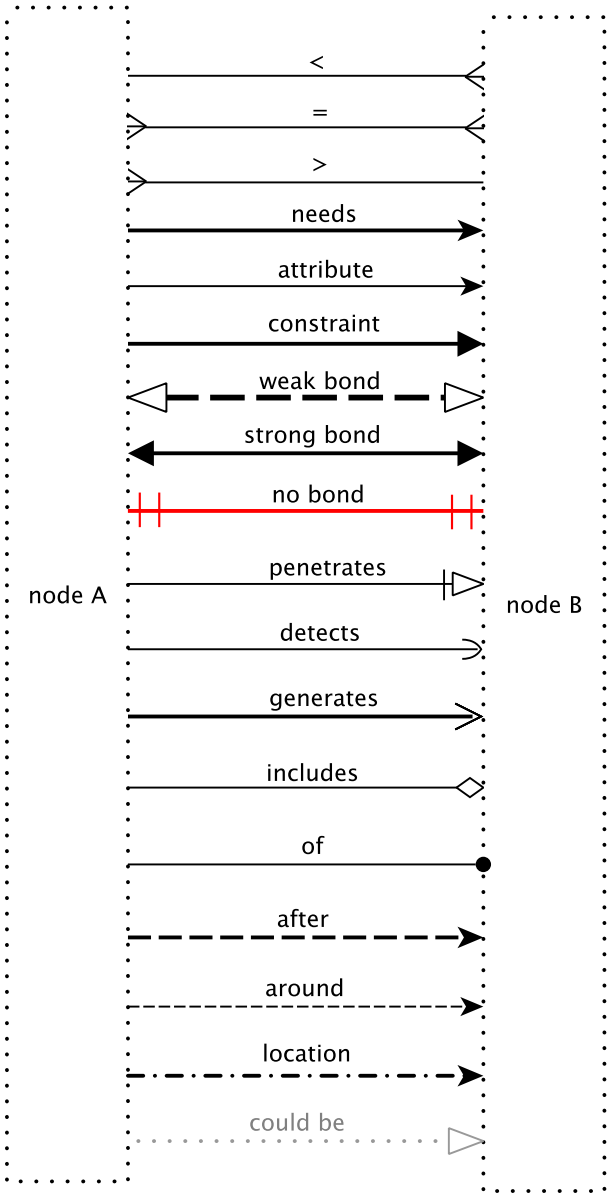

Figure 1: Legend for the meanings of edges that connect the nodes in Figures 2, 3, and 4. **doi:**10.5048/BIO-C.2021.1.f1

such would capture *at least* the above requirements in general content and interdependency. Thus, Figures 2, 3, and 4 reflect a *minimum* of detail logically implied by the purpose, environmental details, existing microbiology of a bacterium, and other such givens.

Figure 2 depicts the root node "bacterium" and the specifications for the control system, the redirection subsystem, and the root of the propulsion system. The shaded box in Figure 3 labeled "alternative motion-to-power schemata" and the several nodes it encloses illustrate several of the potential alternative propulsion schemata. Each trapezoidal node represents a missing large subgraph comprising many potential nodes. Each such subgraph would likely be comparably as intricate as the

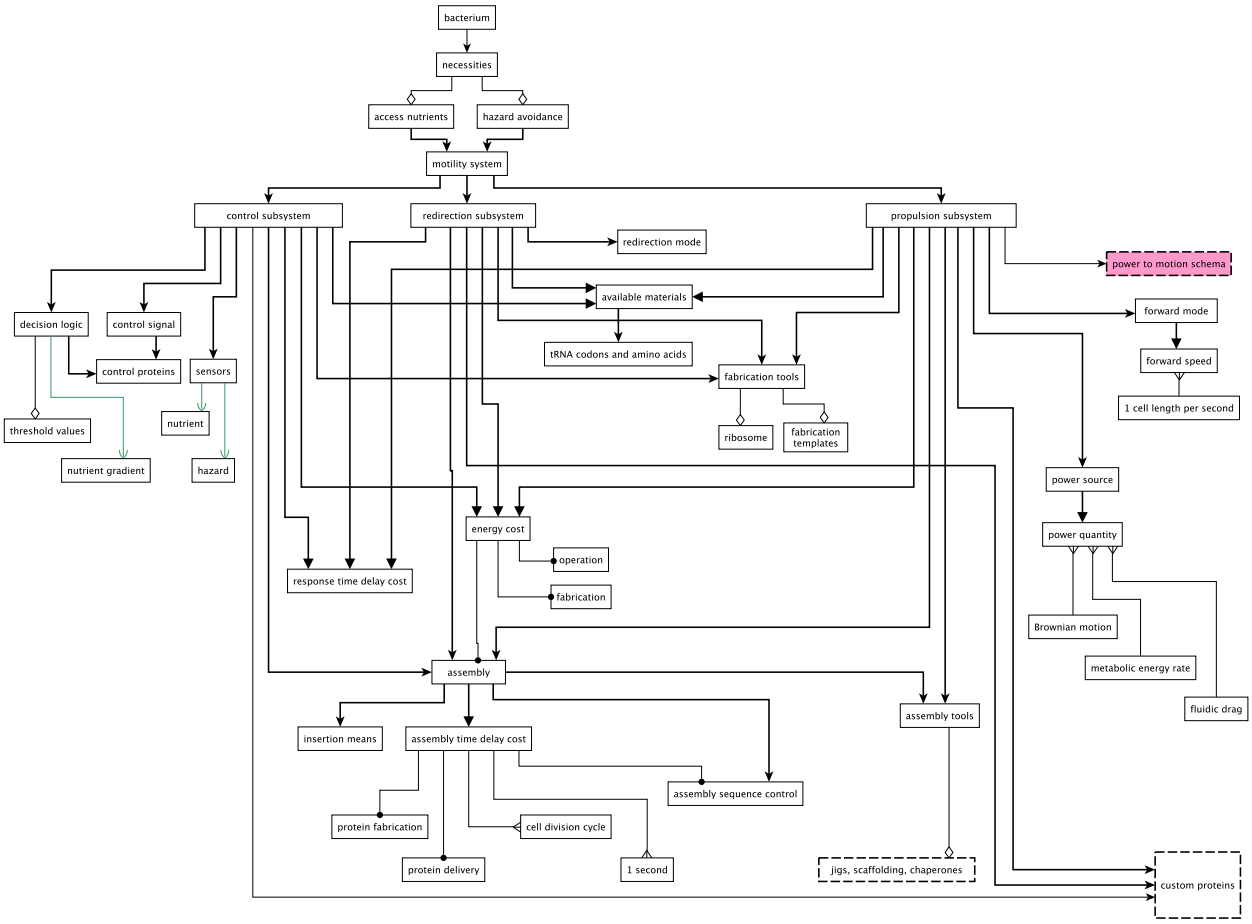

Figure 2: The three subsystems of a bacterial motile system. **doi:**10.5048/BIO-C.2021.1.f2

subgraph in Figure 4 depicting the detailed requirements for "a rotary schema." Each trapezoidal node could have been elaborated for further interest, but that would be a distraction from the needs of this study. Because the specifications for the rotary propulsion schema will be important later, Figure 4 elaborates only that alternative.

The network clearly depicts the cohesive intricacy of the relationships.

A REQUIREMENTS ONTOLOGY

Table 1 is a formal ontology more or less equivalent to Figures 1 to 4, but instead it is in the form of a list of triples (first three columns). Each triple is in the form subject-relation-object or subject-relation-attribute. The coherent interrelatedness of the triples is not as obvious as in the Figures, but triples are more amenable to computer processing. Some of the entries in the third column ("object or attribute") could be further elaborated, because they are more general objects or attributes than the subjects.

Further, a terse rationale or purpose (fourth column of Table 1) is appended to each triple. This is significant, because it suggests that each entry reflects a purposeful and functional aspect of the specification, not a superfluous embellishment.

The first set of several shaded rows in Table 1 indicates some alternative choices of mechanical power-to-motion schemata. However, only the rotary schema is detailed in the subsequent rows. If the other schemata had been elaborated instead, they would have been followed by a plethora of their own distinctive ontology triples. Those details surely would have constituted intricacy, specificity, and coherence comparable to that of the rotary schema.

The second set of several shaded rows in Table 1 indicates the implied details for the optional case where multiple propulsion subsystems are intended to be present in a single bacterium. Under that additional requirement, the torque axis redirector subassembly would be needed so that all the propulsion subsystems could cooperate to propel the bacterium in the same coordinated direction. The redirector would not be necessary where there was only one flagellum in the bacterium. In either case, the specified bacterial motility system would be *irreducibly* complex, but the system with the torque axis redirector subassembly would be one element more complex.

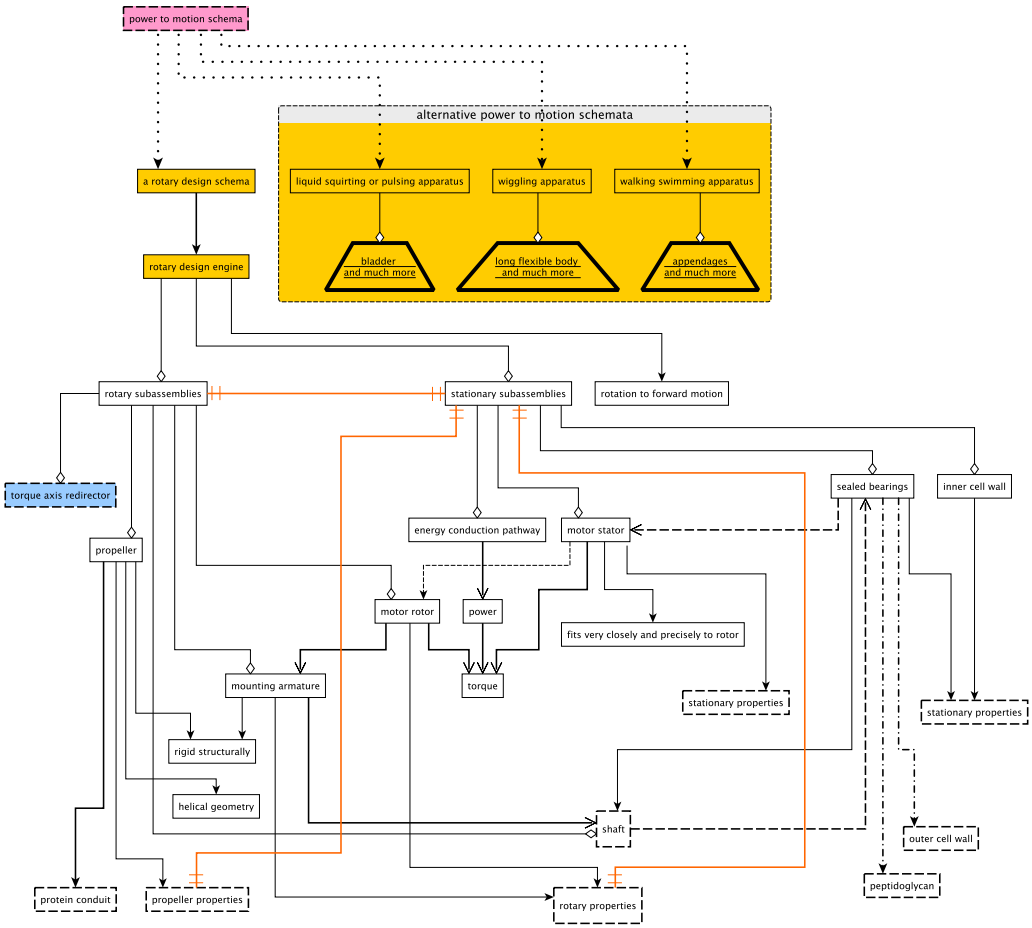

Figure 3: The propulsion part of a bacterial motile system. **doi:**10.5048/BIO-C.2021.1.f3

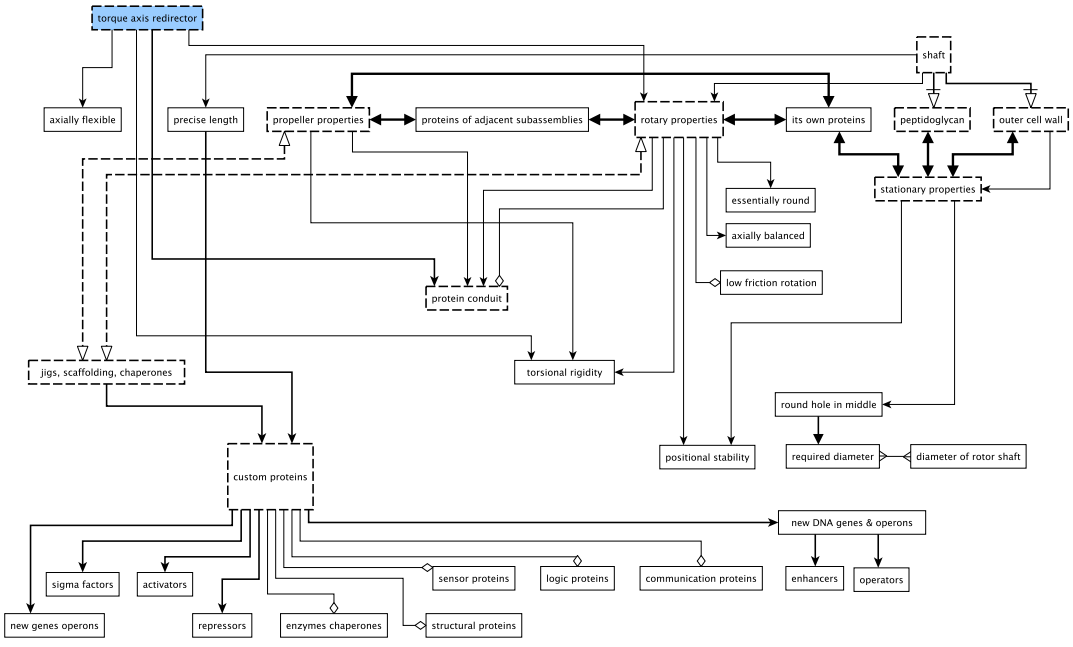

Figure 4: Details of a rotary schema for a bacterial motile system. **doi:**10.5048/BIO-C.2021.1.f4

Table 1: A specification ontology for a bacterial motility subsystem

Subject	Relation	Object	Rationale
bacterium	attribute	necessities	by observation
necessities	includes	access nutrients	for survival
necessities	includes	hazard avoidance	for survival
access nutrients	needs	motility system	to change location
hazard avoidance	needs	motility system	to support escape
motility system	needs	control subsystem	to choose to stay or flee
motility system	needs	propulsion subsystem	to support search
motility system	needs	redirection subsystem	to change location
control subsystem	needs	assembly	to detect whether to stay or flee
propulsion subsystem	needs	assembly	to detect whether to stay or flee
redirection subsystem	needs	assembly	to detect whether to stay or flee
control subsystem	needs	sensors	to detect whether to stay or flee
control subsystem	needs	decision logic	to decide whether to stay or flee
control subsystem	needs	control signal	to control search
sensors	detect	nutrient	to detect nourishment
sensors	detect	hazard	to detect hazards
decision logic	includes	threshold values	to implement decision logic
decision logic	detect	nutrient gradient	to implement hysteresis
decision logic	needs	control proteins	to implement decision logic
control signal	needs	control proteins	to implement control signal
control subsystem	constraint	energy cost	to limit energy usage
propulsion subsystem	constraint	energy cost	to limit energy usage
redirection subsystem	constraint	energy cost	to limit energy usage
energy cost	of	fabrication	to limit fabrication energy
energy cost	of	assembly	to limit assembly energy
energy cost	of	operation	to limit operational energy
control subsystem	constraint	response time delay cost	to limit response time
propulsion subsystem	constraint	response time delay cost	to limit response time
redirection subsystem	constraint	response time delay cost	to limit response time
assembly	needs	assembly sequence control	to order parts delivery, insertion
assembly	needs	insertion means	to position, insert components
assembly	constraint	assembly time delay cost	to limit assembly time
assembly time delay cost	of	protein fabrication	to limit assembly time
assembly time delay cost	of	protein delivery	to limit assembly time
assembly time delay cost	of	assembly sequence control	to limit assembly time
assembly time delay cost	<	cell division cycle	to limit assembly time
assembly time delay cost	<	1 second	to limit response time
propulsion subsystem	constraint	forward speed	to optimize motility
forward speed	>	1 cell length per second	to set speed minimum
control subsystem	needs	fabrication tools	to fabricate components
propulsion subsystem	needs	fabrication tools	to fabricate components
propulsion subsystem	needs	assembly tools	to assemble components

Table 1 (continued)

Subject	Relation	Object	Rationale
redirection subsystem	needs	fabrication tools	to fabricate components
fabrication tools	includes	ribosome	to fabricate components
fabrication tools	includes	fabrication templates	to fabricate components
assembly tools	includes	jigs, scaffolding, chaperones	to facilitate assembly
jigs, scaffolding, chaperones	needs	custom proteins	to facilitate assembly
control subsystem	constraint	available materials	to fabricate components
propulsion subsystem	constraint	available materials	to fabricate components
redirection subsystem	constraint	available materials	to fabricate components
available materials	needs	tRNA codons and amino acids	to utilize cell's normal materials
control subsystem	needs	custom proteins	to implement new functionality
propulsion subsystem	needs	custom proteins	to implement new functionality
redirection subsystem	needs	custom proteins	to implement new functionality
custom proteins	needs	new genes operons	to sequence protein fabrication
new DNA genes operons	needs	enhancers	to initiate operon transcription
new DNA genes operons	needs	operators	to initiate operon transcription
custom proteins	needs	sigma factors	to initiate operon transcription
custom proteins	needs	activators	to initiate operon transcription
custom proteins	needs	repressors	to inhibit operon transcription
custom proteins	includes	enzymes, chaperones	to aid in assembly
custom proteins	includes	structural proteins	to be constituents for propulsion
custom proteins	includes	sensor proteins	to be sensors
custom proteins	includes	logic proteins	to provide control functionality
custom proteins	includes	communication proteins	to control motility
custom proteins	needs	new DNA genes operons	for protein fabrication
propulsion subsystem	needs	motility mode	to determine forward motion, redirection, and/or stop
power source	constraint	power quantity	to specify the power needed
power quantity	>	fluidic drag	to specify the power minimum
power quantity	>	Brownian motion	to specify the power minimum
power quantity	<	metabolic energy rate	to limit the power needed
propulsion subsystem	needs	power to motion schema	to convert power into motion
propulsion subsystem	needs	power source	to power propulsion
power-to-motion schema	could be	liquid squirting or pulsing apparatus	like a jellyfish or rocket
liquid-squirting or pulsing apparatus	includes	bladder and other subassemblies	to convert power into motion
power-to-motion schema	could be	wiggling apparatus	like a sperm or fish
wiggling apparatus	includes	long flexible body and other subassemblies	to convert power into motion
power-to-motion schema	could be	walking swimming apparatus	like a crab, turtle, or frog
walking-swimming apparatus	includes	appendages and other subassemblies	to convert power into motion
power-to-motion schema	could be	a rotary design schema	like a motorboat
rotary design schema	includes	rotary design engine	
rotary design engine	attribute	converts rotation to forward motion	to enable forward motion from rotation

Table 1 (continued)

Subject	Relation	Object	Rationale
rotary design engine	includes	rotary subassemblies	to generate and convey the rotary torque
rotary design engine	includes	stationary subassemblies	to stabilize the rotary subassemblies; to participate in the generation of rotary motion
rotary properties	includes	low friction rotation	to provide sufficient efficiency
rotary properties	includes	protein conduit	to provide assembly channel for internal delivery of proteins
rotary properties	attribute	axially balanced	to provide sufficient efficiency
rotary properties	attribute	torsional rigidity	to transfer torque efficiently
rotary properties	attribute	essentially round	to provide low friction; to act with seals to prevent passage of material past membranes
rotary properties	attribute	positional stability	to provide sufficient efficiency
rotary properties	not bound	stationary subassembly	to provide sufficient efficiency
rotary properties	bound	its own proteins	to form a stable subassembly
rotary properties	bound	proteins of adjacent subassemblies	to form a stable subsystem
rotary properties	weak binding	jig, scaffolding,- and chaperone proteins	to form temporary binds to assembly tools
propeller properties	attribute	protein conduit	to provide assembly channel for internal delivery of proteins
propeller properties	attribute	torsional rigidity	to allow rotation
propeller properties	not bound	stationary subassemblies	to allow rotation
propeller properties	bound	its own proteins	to form a stable subassembly
propeller properties	bound	proteins of adjacent subassemblies	to form a stable subassembly
propeller properties	weak binding	jigs, scaffolding, chaperones	to aid in assembly
rotary subassemblies	includes	mounting armature	to be a mounting structure
mounting armature	attribute	rigid structurally	to transfer torque efficiently
mounting armature	torques	shaft	to transfer torque efficiently
mounting armature	attribute	rotary properties	to be efficient
rotary subassemblies	includes	motor rotor	to be torque generator
motor rotor	generates	torque	to generate torque with stator
motor rotor	torques	mounting armature	to transfer torque efficiently
motor rotor	attribute	rotary properties	to be efficient
rotary subassemblies	includes	shaft	to transfer torque from rotor through the cell wall to the exterior propeller
shaft	penetrates	peptidoglycan	to transfer torque from rotor through the cell wall to the exterior propeller
shaft	penetrates	outer cell wall	to transfer torque from rotor through the cell wall to the exterior propeller
shaft	attribute	precise length	to be long enough to reach cell exterior; not too long
precise length	needs	custom proteins	to specifically do a measurement
rotary properties	attribute	protein conduit	so component proteins can pass to assembly site
shaft	attribute	rotary properties	to be efficient
shaft	torques	torque axis redirector	to convey torque to the redirector
rotary subassemblies	includes	torque axis redirector	to redirect the torque axis
torque axis redirector	attribute	torsionally rigid	to transfer torque to propeller

Table 1 (continued)

Subject	Relation	Object	Rationale
torque axis redirector	attribute	axially flexible	to redirect the torque axis
torque axis redirector	attribute	rotary properties	to be efficient
torque axis redirector	needs	protein conduit	so component proteins can pass to assembly site
rotary subassemblies	includes	propeller	to provide forward motion
propeller	attribute	helical geometry	to convert rotation to forward motion
propeller	attribute	rigid structurally	to maintain helical shape
propeller	attribute	propeller properties	to be efficient and form propeller
propeller	needs	protein conduit	so component proteins can pass to assembly site
a rotary design engine	needs	stationary subassemblies	to stabilize the rotary subassemblies; to help generate rotation; to form seals in membranes
stationary properties	bound	peptidoglycan	to transfer counter torque to cell body
stationary properties	bound	outer cell wall	to transfer counter torque to cell body
stationary properties	attribute	positional stability	to stabilize the rotary subassemblies
stationary properties	attribute	round hole in middle	to provide low friction; to act with seals to prevent passage of material past membranes
round hole in middle	constraint	required diameter	to prevent passage of material past the subassembly and motor shaft
required diameter	=	diameter of rotor shaft	to prevent passage of material past the subassembly and motor shaft
stationary properties	bound	inner cell wall	to be firmly held in place
stationary properties	not bound	cannot bind to any rotary subassembly	to provide sufficient efficiency
stationary properties	bound	its own proteins	to form a stable subassembly
stationary subassemblies	includes	inner cell wall	to transmit counter-torque to cell body
inner cell wall	attribute	stationary properties	to promote efficiency
outer cell wall	attribute	stationary properties	to promote efficiency
stationary subassemblies	includes	sealed bearings	to transfer torque to cell exterior
sealed bearings	location	peptidoglycan	to prevent passage of material past the bearing; to stabilize rotational subassemblies
sealed bearings	location	outer cell wall	to prevent passage of material past the bearing; to stabilize rotational subassemblies
sealed bearings	around	shaft	to prevent passage of material past the bearing; to stabilize rotational subassemblies
shaft	before	sealed bearings	to locate where the bearings need to assemble
sealed bearings	attribute	stationary properties	to promote efficiency
stationary subassemblies	includes	motor stator	to generate rotation along with motor rotor
motor stator	around	motor rotor	to generate torque with rotor
motor stator	attribute	fits very closely and precisely to rotor	to generate torque with rotor
sealed bearings	before	motor stator	to be located properly with respect to motor rotor
motor stator	generates	torque	to generate torque with rotor
motor stator	attribute	stationary properties	to promote efficiency
stationary subassemblies	includes	energy conduction pathway	to supply power
energy conduction pathway	generates	power	to convert energy to torque
power	generates	torque	to actively power the rotational torque

OBSERVATIONS

Regarding the foregoing derivation of requirements and Figures 2 to 4, we see intricate coherence which is essentially irreducible. It is hard to imagine that a motility system (comprising control, propulsion, and redirection subsystems) could function at all without each of those details present.

Current evolutionary biology proposes that the flagellum could have been "engineered" naturalistically by cumulative mutations, by horizontal gene transfer, by gene duplication, by co-option of existing organelles, by self-organization, or by some combination thereof [10, p. 210]. See the summary and references by Finn Pond [11]. Yet to date, no scenario in substantive detail exists for how such an intricate propulsion system could have evolved naturalistically piece by piece. Can any partial implementation of a motility system be even slightly advantageous to a bacterium? Examples of a partial system might lack sensors, lack decision logic, lack control messages, lack a rotor or stator, lack sealed bearings, lack a rod, lack a propeller, or lack redirection means. Would such partial systems be preserved long enough for additional cooperating components to evolve?

Further observations will conclude Parts 2 and 3. They will include suggestions for further research into the molecular details of proteins composing the bacterial flagellum, as detailed in Part 2.

ACKNOWLEDGMENTS

The reviewers provided valuable insights and corrections. This article is far better because of those comments, which are truly appreciated.

REFERENCES

1. Alon U (2007) An Introduction to Systems Biology: Design Principles of Biological Circuits. Chapman & Hall/CRC (Boca Raton).
2. Conrad E et al. (2020) Waterfall Model. An auto-generated Science Direct overview of the literature. https://www.sciencedirect.com/topics/computer-science/waterfall-model. [Cited: February 19, 2021].
3. JavaTPoint (2020) Waterfall Model. https://www.javatpoint.com/software-engineering-waterfall-model. [Cited: February 19, 2021].
4. Dennis A et al. (2015) Systems Analysis and Design: An Object-Oriented Approach with UML. John Wiley (Hoboken, NJ).
5. Sylvia W (2021) How to Write an Engineering Requirements Document. Fictiv (San Francisco). https://www.fictiv.com/articles/how-to-write-an-engineering-requirements-document [Cited: February 23, 2021].
6. Elgabry O (2016) Requirements Engineering – Requirements Specification (Part 3). https://medium.com/omarelgabrys-blog/requirements-engineering-elicitation-analysis-part-5-2dd9cffafae8. [Cited: February 19, 2021].
7. Grady J (2014) System Requirements Analysis, 2nd Edition. Elsevier (Waltham, MA).
8. IEEE Standards Association (2018) ISO/IEC/IEEE International Standard: Systems and software engineering—Life cycle processes—Requirements engineering. Institute of Electrical and Electronic Engineers, Inc. (New York). https://standards.ieee.org/standard/29148-2018.html
9. Purcell EM (1977) Life at low Reynolds number. American Journal of Physics 45:3–11. doi:org/10.1119/1.10903
10. Liu R (2009) Origin and Evolution of the Bacterial Flagellar System. Chapter 13 of Pili and Flagella: Current Research and Future Trends. Caister Academic Press (Norfolk, VA) 207–214.
11. Pond F (2006) The Evolution of Biological Complexity. Reports of the National Center for Science Education 26(3). https://ncse.ngo/evolution-biological-complexity