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Research Article

An Engineering Perspective on the Bacterial Flagellum: Part 3—Observations

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Abstract

The flagellum is the organelle imparting motility to common bacteria. This paper, the third of three, takes a *systems engineering* and *systems biology* perspective on the bacterial flagellum. The first paper (Part 1 of the series) provided a *constructive* or *top-down* view from a *systems engineering* viewpoint. It detailed the typical environment, the purpose, the required existing and new resources, the necessary functional requirements, the various constraints, the control means, and the self-assembly for any kind of bacterial motility organelle. The specification of these requirements was intended to be independent of knowledge about the actual flagellum. A converse approach was detailed in the second paper (Part 2 of the series). It was an *analytical* or *bottom-up* view, which discussed the known 40+ protein components and the observed and inferred structure, control, and assembly of a typical bacterial flagellum. This cellular subsystem is well-researched. Much of that research was reviewed in Part 2 from a systems biology viewpoint, including the chemotaxis feedback control system. Part 2 included a very detailed dependency graph of the orchestrated assembly not found elsewhere. This third paper (Part 3) concludes the three-part study with original observations. The observations include an ontology of the exceedingly specific protein binding relationships in the flagellum. The latter observation is new and significant and suggests research to further elaborate the details of the molecular configurations of the proteins. Part 3 also compares the independent constructive and analytical views, which correlate well. Finally, it is suggested that a motility organelle of this scope and scale seems profoundly unlikely to naturally evolve in the absence of foresight and mindful intent.

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INTRODUCTION

This is the third of three connected papers presenting an engineering perspective on the bacterial flagellum. Part 1 presented a "constructive" view [1]; Part 2 presented an "analytical view" [2], and this, Part 3, presents pertinent observations and conclusions.

The flagellum is the organelle imparting motility to common bacteria. The systems perspective on the bacterial flagellum detailed in in Parts 1 and 2 studied 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 was also studied. This study took two essentially independent approaches, a *constructive* approach in Part 1, and an *analytical* approach in Part 2.

The *constructive* approach was a *top-down specification*. That is, it started with specifying the purpose of a generic 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 derived the logically necessary functional requirements, the constraints, the assembly needs, and the hierarchical relationships within the functionality. The functionality needed a control subsystem to properly direct the operation of a propulsion subsystem. Those functional requirements and constraints then suggested a few—and only a few—viable implementation schemata for a bacterial propulsion system. The entailed details of one schema were then set forth. A sincere attempt was made to keep the elaboration of this constructive approach as independent as possible from knowledge of the actual flagellar structure. The result was a complex ontology specifying the general interrelated, coherent

requirements for any motility subsystem for a bacterium.

The *analytical* approach was a *bottom-up analysis* accumulated from the cited literature. The approach started with the constituent proteins, observed structure, assembly, and resultant behavior of an archetypical bacterium. This included its chemotaxis control subsystem. Such knowledge has been acquired by microscopic observation, by gene sequencing, by disabling component proteins (gene "knock-out" experiments), and by other experimental methods. Higher-level organization, functionality, mechanism, and assembly orchestration are hypothesized and inferred from those basic low-level details, but much of the overall understanding still remains unclear. A very detailed dependency graph showed the orchestration of the assembly of those components into a flagellum. The specifics related mostly to the two most studied species of bacteria: *Salmonella enterica* for assembly details of the flagellum; *Escherichia coli* for chemotaxis as the operational control means.

This Part 3 will first document the tightly constrained pairwise relationships among the several dozen types of flagellar proteins and the well-defined subassemblies composed of those proteins. This observation is derived from the just-discussed analytical view, but appears to be a fresh, significant observation suggesting further research.

Next, the constructive and analytic approaches will be compared. This is a typical engineering step, because engineers regularly design and specify systems top-down, but they construct those systems bottom-up. So, the resulting bottom-up implemented system will be evaluated against the top-down specification.

Finally, the paper will offer additional observations from various viewpoints, and a tentative conclusion about the origin of the bacterial flagellum.

EXQUISITE MOLECULAR BINDING OF PROTEINS

Figure 1 depicts the intricate pairwise binding relationships among the various subassemblies of the flagellum. Each subassembly or set of proteins is represented by a rectangular node and comprises a set of proteins or protein complexes named in the node.

The various binding relationships between pairs of subassemblies or sets of proteins are represented by several distinct types of line connecting the nodes. The figure shows five different binding relationships between the nodes. The lines with solid arrowheads represent strong non-covalent bindings, both between the subassemblies and between proteins within a subassembly. A line with hollow arrowheads represents a temporary binding with either a chaperone protein or a scaffolding ("assembly jig") protein complex. A line with a bar at each end means the two subassemblies must be free of any binding or interference. A line with a circle at each end indicates that the proteins (or protein complexes) of a subassembly make up connected segments of a circular annulus or helical segments of a tube. (Roughly speaking, each segment forms one of several segments of an annulus or is one segment of a helix forming a tube.) A line with a solid square on each end indicates a tight tolerance. In the case where one node is the lumen, the line connecting it represents the required geometrical tolerance of the diameter of its central channel, which is formed by the subassembly named in the other node. In the case where both nodes are subassemblies, the line with solid squares at each end indicates that the two subassemblies must fit so closely that no large molecule can pass between the subassemblies. Nevertheless, those assemblies must not bind and must move freely with respect to each other.

The lines in Figure 1 represent highly specific properties of the proteins composing the various subassemblies.

First, the molecular structure of the proteins forming an annulus or tube must have the property that the proteins have binding sites which firmly connect the proteins so they sequentially abut. In doing so, each protein must effectively form an N-degree arc. That is a very specific geometrical and amino acid sequence property for a folded protein, which only extremely rare protein configurations could meet. Further, for proteins forming a tube, each round of the helical, coiled-rope-like, end-to-end connected proteins, and each loop of the helix binds to the preceding and succeeding loops to form a stable tube, and thus can efficiently transmit torque by being tangentially rigid (see [3], pp. 562-567). In other words, many of the proteins must bind to four other adjoining proteins: the proteins fore and aft along each loop of the helix, and the proteins of the preceding proximal and succeeding loops around the helix. The neighboring proteins may be similar (such as within the filament) or dissimilar (where one subassembly binds to the next). That is, the proteins of each subassembly must firmly bind with the proteins of at least one other subassembly. These binding properties require rigorous, very specific requirements on the folded chain of amino acid residues of those proteins, that is, the aligned locations of non-covalent binding between two similar or dissimilar proteins.

Second, the helical nature of a tube especially simplifies its automated assembly by means of its scaffolding proteins, whereas forming stacked rings might require more complex scaffolding proteins to close one ring and then initiate the next. In contrast, building a helix is much like adding links to a continuous chain that coils one round on top of the other as it grows. Kato et al. [4] have revealed the cross-linking among the proteins of the "supercoiled" flagellar hook and show why the hook is longitudinally flexible while maintaining torsional rigidity.

Third, a so-called scaffolding subassembly needs to have a special temporary binding relationship with the subassembly it helps build. This property is a further rigorous requirement. A similar property relates to the several chaperone proteins used during assembly of the flagellum.

Fourth, certain pairs of subassemblies must have no attraction as shown by the "must never bind" lines in Figure 1. For example, the proximal rod must freely rotate inside the P ring. That property strictly limits the amino acid configuration in the proteins involved. It further implies that the rod must be nearly circular, and so must the "donut hole" of the P ring (in

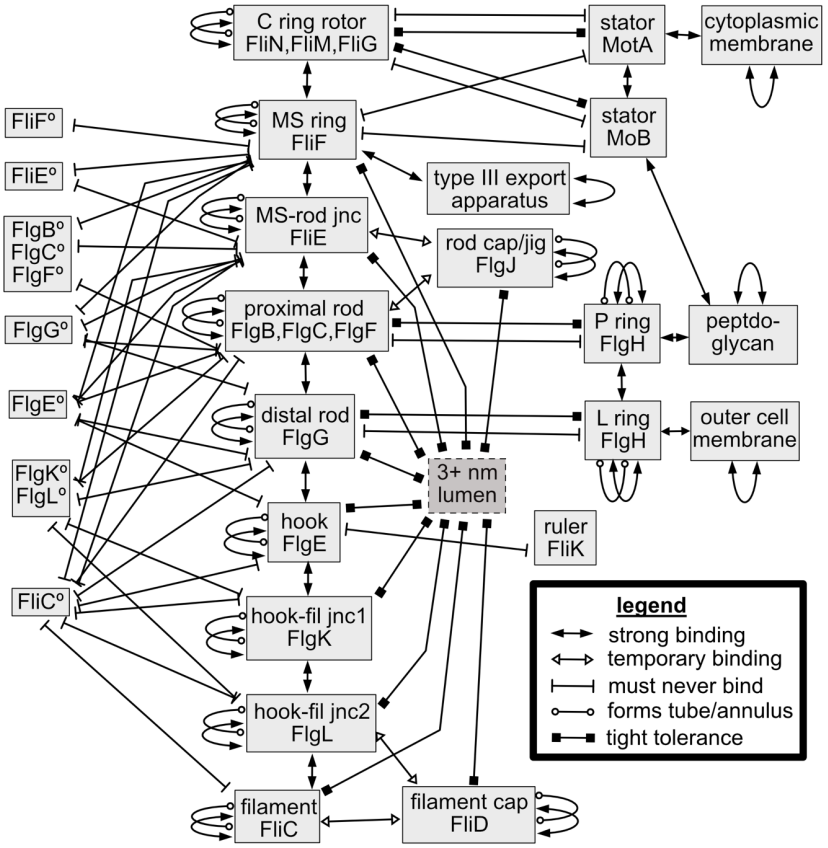

Figure 1: Binding and geometrical properties of the proteins. **doi:**10.5048/BIO-C.2021.3.f1.

cross-section) through which the rod fits with very small tolerance. While the proximal rod must rotate with little friction, the P ring and proximal rod nevertheless must be so close to each other that there is no "leakage" through the space between them. Similar observations hold for the L ring and distal rod and for the stator and rotor subassemblies. That is, the inner diameters of the "donut hole" of the rings must very closely match the outer diameters of the rod subassemblies. The circularity requirements and the tight tolerances are yet two more geometrical properties needing to be met by the constituent proteins and the way they self-coalesce.

Fifth, in addition to the tight tolerance just mentioned, there is a strict tolerance on the diameter of the lumen formed by all the rotary subassemblies. Its minimum diameter must be at least 3 nm so that (unfolded) proteins can pass through it during assembly. Its maximum diameter is also limited. If it were several times larger, the annuli and tubes would require more segment proteins (or larger proteins)—at a cost to the cell.

Sixth, each unfolded—but not yet situated—structural protein that is chaperoned through the lumen must not bind with any of the folded proteins that form the lumen until it reaches its destination. These unfolded proteins are represented by the leftmost column of nodes. Each of the unfolded proteins named in the leftmost nodes is denoted with the symbol °. They must traverse at least a part of the lumen. For example, FliC° must traverse the whole lumen, but FlgG° will not traverse the lumen past the distal rod.

Further, the research literature is silent about forces or mechanisms that may be involved in directing and propelling those unfolded proteins distally through the lumen. If it is some capillary-type action or simply diffusion, why is it unidirectional? Note that (at least at the macro scale) the diffusion rate through a small tube is low and gets even lower as the tube lengthens.

The six properties just discussed must all be present, so the already extremely rare protein configurations of the first property are even more rigorously restricted by the other required properties.

COMPARISON OF THE CONSTRUCTIVE AND ANALYTIC APPROACHES

The following correlates the Part 1 constructive perspective with the Part 2 analytical or reductionist perspective.

Tables 1 through 3 list the top-down, logically-derived constructive functional requirements—a specification—alongside the actual composition of a typical flagellum, including its control and redirection features. Table 4 shows the correlation between the top-down specified assembly functions and the aspects of the actual assembly process.

Table 1: Comparison of top-down specification with bottom-up analysis: *Control*

top-down specification	bottom-up analysis
required **operational** functionality	archetypical implementation
control subsystem	chemotaxis system
sensors	Tsar for serine
sensors	Tar for maltose aspartate
sensors	Trg for galactose ribose
sensors	Tap for dipeptides, *pyrimidines*
sensors	Aer for oxygen
conditional logic	methylation level of CheR, CheB
control signals	CheY, CheZ interact with rotor switch

Table 2: Comparison of top-down specification with bottom-up analysis: *Propulsion*

top-down specification	bottom-up analysis
required *propulsion* functions	archetypical implementation
for all schemata	(see below)
power source	ion (H+ or sodium) gradient
power-to-motion means	rotary electric motor
external component(s)	rod end, hook, filament
foundation/substrate	inner/outer membranes, peptidoglycan
rotary schema: rotary subsystem	(see below)
armature	MS ring: FliF proteins
motor rotor	C ring: FliG, FliM, FliN
shaft	rod: FlgB, FlgC, FlgF; FlgG
(axial bend, if side mount)	hook: FlgE
helical propeller	filament: FliC, FliD (cap)
(possible adaptors)	hook-filament: FliE, FlgK, FlgL, FlgM
seals-bearings	P ring & L ring: FlgI, FlgH
motor stator	MotA, MotB
constraints	(see below)
custom proteins	about 30+10
energy (low enough to be worthwhile)	(unknown, but apparently) low enough
response time < 1 sec to redirect	can reverse torque in less than 1 rotation
speed > 1 cell length / sec	much faster
binds to copies of self	flagellum is rigid and stable
binds to two other subassemblies	flagellum is rigid and stable
binds to chaperone	this clearly works
folded in place	rod, hook, filament proteins

Table 3: Comparison of top-down specification and bottom-up analysis: *Redirection*

top-down specification	bottom-up analysis
required *redirection* functions	archetypical implementation
redirection subsystem	clockwise rotation → tumbling
switch	part of C ring
constraints:	(see below)
responsive to signals	CheY, CheZ interact with rotor switch

Table 4: Comparison of top-down specification and bottom-up analysis: *Assembly*

top-down specification	bottom-up analysis
required *assembly* functions	archetypical implementation
tools	existing gene expression machinery
templates	class 1, 2, and 3 operons, tRNA
jigs, scaffolds	FlgJ, FlgD
parts list	class 1, 2, and 3 operons
parts delivery	Sec pathway and export apparatus: FlhA,FlhB,FliO,FliP,FliQ,FliR,FliI,FliH (and diffusion)
parts placement	occurs, but not detailed in the research literature
parts insertion	possibly electrostatic attraction & non-covalent binding (not detailed in the research literature)

Clearly there is high correlation between the top-down and bottom-up perspective. What is the implication of this correlation? It suggests the configuration of the flagellum is purposeful.

FURTHER OBSERVATIONS

An Inventor's Observation

An experienced inventor or patent agent would realize that most apparatus and process patents are far less intricate than the flagellum, its operation, and its assembly processes.[1] Yet purposeful design is present in even the simplest issued patent [5]. If purposeful design is publicly recognizable, in the case of even the simplest patents, how much more so must it be recognizable in systems as complex as the flagellum! As Minnich and Meyer have stated, "In any other context we would immediately recognize such systems as the product of very intelligent engineering" (see [6], p. 302).

Note that many patents have been awarded simply for novel proteins. Yet the design of a single protein involves far less intellectual content and originality than would be required to

[1] Further, the complexity of the flagellum is dwarfed by the complexity of the whole bacterium.

design a coherent complex of proteins self-assembling into an organelle. This exposes a certain irony: the intellectual input of inventors is recognized in a human-designed protein but not in a natural protein—or in a coherent *subsystem* of proteins composing an organelle like the flagellum.

An Engineer's Observation

Second, an experienced engineer would fully appreciate the mental effort, insightful creativity, inventive genius, and foresight that even a rather simple device requires. It begins with observing a need or problem, implying purposeful insight. That is followed by identifying the available resources (materials, tools, existing parts), necessary functions, normal environment, physical constraints, and so on. Then such factual input is followed by one or more design schemata. While numerous design options may be conceived, a very few fully comply with the foregoing requirements and constraints. This whole process requires significant mental effort and is far from trivial or accidental. Nevertheless, all that *abstract specification* still does not instantiate a physical entity. A series of one or more *prototypes* must be physically constructed. A prototype is then *tested for compliance* for substantially satisfying the need or solving the original problem and, more specifically, all the stated and logically derived requirements. All that applies to the flagellum, as the foregoing discussion has shown.

A first prototype may conveniently be a stripped-down version of a full, intended solution. But it still must operate well enough to be evaluated against at least some of the requirements. Obviously, a string of increasingly intricate or increasingly compliant prototypes is a kind of "evolution," but it is naïve to consider that as a *blind* trial-and-error search for a solution. Although Edison tried many materials before he produced a successful light bulb, even those candidate materials were an intelligently chosen subset of many, many more potential materials. For example, the material had to conduct electricity but have some resistance. Similarly, Microsoft, Apple, or Google would never—could never—produce specified software by any blind search. The combinations of software instructions are so exponentially vast that even a most trivial program meeting a trivial specification could never be "discovered" in this way.

The testing of the prototype(s), while benefiting from thoughtful design, can be automated to validate or to invalidate each putative prototype. Yet testing does not *fix* an invalid design. Similarly, biological natural selection is "automated" and is quite *credible* and *demonstrable*, but it is no more than a *passive filter* for the "testing" of an organism's viability; it creates nothing new. Indeed, so-called "selection pressure" is not a measurable physical "entity" or "force," but rather is a *phenomenon*, simply a description of differential survival devoid of any innovation.

What seems *incredible* is that mindless, random mutations could ever innovate and instantiate any coherent, intricate functionality beyond trivial modifications to existing functionality [7]. Were a string of such stepwise mutations to occur, at least some functionality must obviously be maintained at each step, or else a nascent incomplete function or organelle would not be conserved intact long enough for later mutations. The odds are massively against the chance formation of one or more ultra-lucky configurations of an interacting complex of new proteins which properly bind together, or even simply one custom, functional, folded protein [8]. Further, the notion of co-opting existing proteins (or mutations thereof) presumes that such proteins possess the bevy of characteristics detailed above, such as matching binding sites, not binding to each other in many cases, precise dimensions relative to each other, coordinated functionality, control of fabrication and assembly order, and appropriate stoichiometry.

An Evolutionist's Observation

Aizawa honestly admits the following:

> Since the flagellum is so well designed and beautifully constructed by an ordered assembly pathway, even I, who am not a creationist, get an awe-inspiring feeling from its 'divine' beauty…. However, if the flagellum has evolved from a primitive form, where are the remnants of its ancestor? Why don't we see any intermediate or simpler forms of flagella than what they are today? How was it possible that the flagella have evolved without leaving traces in history? (see [9], p. 91)

In the light of that admission, how can he or any other evolutionary theorist so assuredly claim (see [9], p. 96) that the flagellum's assembly pathway "has been streamlined by evolution to minimize the time of the assembly process" and that the flagellum "has acquired its beauty by evolving such a sophisticated, efficient machine"?

Indeed, Aizawa's three questions above still stand unanswered. Further, how can the flagellum have evolved step by step through nonfunctional, partially complete structures? How could a partial subsystem be functional or survive unscathed through enough generations to become a functional final form? Could a precursor flagellum with an assembly time much slower than the cell reproduction time survive? Which subassemblies can be missing and yet have partial functionality? Perhaps the hook for a bacterium with a single flagellum. But which other subassembly? And what about the coevolution of a required control subsystem?

Aizawa attributes optimization to evolution. However, most optimization algorithms only find optimal values for a limited, pre-specified set of parameters. Such algorithms only optimize the few logically specified parameters; they do not suggest new parameters to optimize and certainly do not suggest a new feature for the object being optimized. Further, without intelligently chosen, appropriate starting values—or a more sophisticated, robust algorithm—simpler algorithms often converge to local, suboptimal values, *if the algorithm converges to any value at all*. This is especially true as the number of parameters to be optimized increases.

What about evolutionary computer algorithms, which claim to randomly generate new parameters? Marks et al. (see [10], pp. 187-223) show that even these algorithms (such as EV and

Avida) fare no better unless programmed-in, purpose-driven guidance is implicit in the search for a new solution to a problem. Even so, such algorithms use mathematical models in tailored, non-trivial software—not in live biological systems. They are *conceptual*. Does that transfer to the mindless *physical* world without intelligent input and manipulation?

Attempting to provide evidence of evolution of the flagellum, Liu [11] presents a putative phylogenetic tree of 48 bacteria based on 14 specific flagellar proteins, but it is not clear that the same tree would result if more or other proteins were included. Indeed, Liu presents 24 proteins shared by all flagellar systems. See also Samatey [12]. Merino [13] agrees: "Comparison of the complete genome sequences of flagellated bacteria revealed that flagellar structural proteins are based on an ancient core set of 24 flagella genes that were present in the common ancestor to all Bacteria." Two dozen genes require quite a few innovative origins lacking detailed explanations—origins presumably occurring nearly simultaneously. Further, is there *any* evidence that proteins produced by a smaller combination of those genes have any function? In any case, as noted above, there is yet no trace of flagellar lineage from some simpler, functional motile organelle.

A Molecular Biologist's Observation

The overwhelming observation from this flagellum study, noted in the section on exquisite bindings, is that each of the structural proteins of each of the flagellum's subassemblies requires an extremely precise molecular configuration in order to simultaneously comply with several very specific required properties. First, the copies of the constituent protein(s) of a subassembly must bind tightly to themselves, because at this scale, where Brownian turbulence is dominant, the flagellum must be exceedingly tough and robust and must efficiently transfer torque. Second, the folded geometry of many of the proteins must be curved arcs that lead to the formation of annuli and tubes—binding head-to-tail; those proteins forming tubes must also bind to like proteins in preceding and succeeding turns of the helix. Third, the diameters of the annuli and tubes are critically matched, to form a seal and yet to allow efficient rotation; similarly, the central channel/lumen is critically sized. Fourth, each copy of the constituent proteins of a subassembly must tightly bind to the proteins of the one or two adjacent subassemblies. Note that there is a required coupling adaptor between the flexible shaft and the filament to accomplish the fourth requirement. Such intricate coherence was neither expected nor anticipated [6].

Further, regarding the flexible shaft ("hook"), the constituent proteins must stretch and contract longitudinally as it rotates around a bent axis. Simultaneously, the proteins must be very inflexible transversally. This is a requirement in addition to the above required properties.

Still further, regarding the filament, its constituent proteins are very distinctly constituted so that (1) the filament forms a helix of an appropriate size and (2) it retains a rigid helical shape when rotating counterclockwise but becomes flaccid when rotating clockwise.

A Philosopher's Observation

Attributing the implementation of a bacterial flagellum to neo-Darwinian evolution, where no substantive evidence for such a construction exists, seems like presumptive faith in the magic of very fortuitous co-option and the stepwise mutations of duplicated genes, intercellular gene transfer, exaptations, and *de novo* saltations. It involves a *philosophical* prior commitment to Naturalism. That commitment was displayed in Aizawa's statements above. Further, using homologous proteins to posit an evolutionary tree neither demonstrates that the evolutionary process happened nor obviates alternate explanations (such as intelligent bioengineering).

FUTURE RESEARCH

The future work for a Darwinist, or any other evolutionary theorist, is (1) to provide a *detailed* hypothesis for how all the tightly constrained interlocking coherence described above could have evolved naturalistically under real-world constraints and (2) to show evidence that such a scenario actually transpired in the past.

For the molecular biologist, the control and sequencing of protein fabrication and assembly begs for further elucidation. This would include what controls the stoichiometry of the various subassemblies and how the correct sequence of proteins is gated through the type 3 export apparatus. Further, a study of the binding sites of the proteins to each other and to their immediate neighbors would be a helpful endeavor, the principles of which could have much wider application. This is nontrivial, because there are quite a few protein-protein pair bindings to consider—both those pairs that bind and the pairs that should *not* adhere to each other (lest they impede the assembly process). Even more, how/when/why do flagellar chaperone proteins attach and then detach from the proteins they guard or direct? What controls that?

For the molecular modeller, future work could simulate in detail the geometry and specific binding loci of each pair of bound proteins. How do they become oriented to each other by electrostatic attraction? Can the binding force be estimated? Further simulation might illustrate how the chaperones and scaffolding proteins bind and then release. Lastly, one might model the interface between the L and P ring proteins to illustrate (1) how they together with the rod form a seal which does not allow passage of all but the smallest molecules past their interface and (2) why the rings do not bind with the rod.

For the information scientist, computing the information content (by some measure) in the ontologies and graphical networks of Parts 1 and 2 would be fascinating.

Obviously, the bacterial flagellum has been as widely studied as have many other cell functions and organelles. It would be useful to apply the approaches and level of detail portrayed above to those other organelles. The same approach could be applied to viruses, bacteriophages, gametes/zygotes, and on up to multicellular flora and fauna.

CONCLUSIONS

The above observations derive from all the facts discussed herein; they are objective. Engineers and patent agencies always attribute functional devices—even trivial ones—to some intelligent designer(s), implementor(s), or inventor(s). Meanwhile, the challenge to the evolutionary biological community is to hypothesize some putative *detailed, step-by-step* scenario to explain how the flagellum and its control system was blindly engineered naturalistically. Yet, that would still fall very far short of real evidence that the scenario actually occurred, given real-world constraints. How would portions of a nascent flagellum be protected from degradation while the remainder were yet to be gradually evolved? If some of the requirements discussed above could be omitted, what function would that provide?

These are real questions that demand answers. Yes, these are hard questions, and we surely do not know nearly enough yet to answer them. The challenge is to answer them. Meanwhile, it seems disingenuous to pretend that questions about intelligent causation are irrelevant and inconsequential when so much is already known about the hierarchical assembly, control, and function of the flagellum. Yet the mechanism of the dominant explanatory framework is such a mindless and unimaginative process.

A motility organelle of this scope and scale seems profoundly unlikely to naturally evolve in the absence of foresight and mindful intent.

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