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The biophysicist’s guide to the bacterial flagellar motor

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ABSTRACT
The bacterial flagellar motor (BFM) is a rotary electric nanomachine that drives swimming in a wide variety of bacterial species. There have been many milestones, both theoretical and experimental, that have furthered our understanding of this tiny motor since the first swimming flagellated bacteria was observed. In this article, we review some of these key events, and illustrate how theory and experiment intertwine and inform each other towards a deeper understanding of the BFM’s mechanism. Experimental results have inspired theoreticians to build and update models, while model predictions have served to guide experimental design. This cooperative and mutually beneficial communication is a prime example of the interdisciplinary and open nature of modern scientific research.

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1. Introduction
The cell attached on a slight rise just on the edge of the stomach. It stood on its own and looked out over a broad spread of the Gastrointestinal System. Not a remarkable cell by any means – it was about 2\,\mu\text{m} long, rod-shaped, made of proteins, and had four flagella with rotary motors set in the membrane of a size and proportion which more or less exactly succeeded to stimulate your mind. — Pastiche of The Hitchhiker’s Guide to the Galaxy by Douglas Adams

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Figure 1. Rotation of a bacterium’s flagellum is driven by a rotary motor attached to its base by a flexible hook.

Notes: The rings of the flagellar motor’s basal body are called the rotor. FliG proteins are placed around the periphery of the rotor’s C-ring. These interact with the loops of the stator-units to generate torque and rotate the flagellum. Each stator unit is composed of MotA and MotB proteins, the latter of which attaches the stator to the peptidoglycan layer, allowing for torque generation via the MotA–FliG interaction. A motor maintains up to 11 engaged stator units, depending on the load.

Rotary motors, despite their ubiquity in man-made machinery, are rarely found in the natural world. Only flagellated bacteria are known to incorporate such machinery in their locomotive strategy, making use of the rotary bacterial flagellar motor (BFM) to spin long flagellar filaments that sprout from the cell body (Figure 1). In this review, we provide an overview of the theoretical and experimental steps researchers have taken towards understanding one of the most powerful and efficient machines in existence.

The basal body of the BFM spans the cellular envelope and is comprised of several transmembrane rings that connect to the bacterium’s flagellar filament by a flexible hook. These rings are approximately 45 nm in diameter, containing approximately 25 different proteins. Motor rotation is known to occur via an interaction between one or more membrane-embedded torque-generating stator units and spoke-like proteins along the periphery of the rotor ring.
This interaction is powered by the ion-motive force arising from the transit of ions (protons, in the case of the commonly studied *Escherichia coli* motor) across the cellular membrane. The BFM is remarkable in its ability to efficiently convert the free energy stored in this transmembrane electrochemical gradient into mechanical work: while man-made engines lose significant amounts of energy to heating, the BFM operates at close to 100% efficiency. Rotating at approximately 300 Hz (or 18000 rpm; compare to the upper limit of a typical car engine’s rotational speed of 6000 rpm), the *E. coli* motor can output a power of approximately $1.5 \times 10^5$ pN nm s$^{-1}$ [1] and propel the bacteria at a speed up to 100 µm s$^{-1}$ – that is, up to 100 body lengths per second! The BFM of other species have been shown to rotate several times faster.

The BFM’s fundamental role in bacterial locomotion makes it central for several biological processes, including chemotaxis, surface swarming and biofilm formation. Accordingly, it is one of the best-studied molecular machines, both theoretically and experimentally. However, for a long time, detailed modelling efforts have been stymied by the lack of atomic-level structural information, which is difficult to obtain due to the motor’s size and localization to the membrane. In this review, we chronicle the attempts to clear this hurdle in order to propose mechanically specific, experimentally testable models of the motor’s torque-generating mechanism.

Our primary aim is to outline the prominent experimental and theoretical milestones in the study of the BFM, highlighting how each approach has built upon and informed the other (for a summary of these milestones, see Figure 2). We refer the interested reader to several excellent review articles for further information on the BFM generally [2–5], or regarding more specific topics: on models of the BFM [6]; on BFM structure [7–10]; on dynamics of the BFM stator [11,12]; on the sodium-driven motor [13]; on diversity of flagellar motors across bacterial species [14]; and on BFM switching in chemotaxis [1].

## 2. Experimental milestones

The first swimming bacterial cells were observed in the seventeenth century by Antonie van Leeuwenhoek. But the powerhouse behind this locomotion, the BFM, remained a mystery. Too tiny to be observed easily using optical microscopes, the flagellar filament and the motor that spins it went unnoticed for 300 years. Starting from the mid-1900s, we outline here several important experimental leaps that have led us to our current understanding of the BFM.

### 2.1. Electron microscopy images (1971)

Understanding, or even imagining, how something works is, not impossible, but really, really improbable, if it has never been seen. So, predictably, the first investigation into the flagellar machinery concerned its structure: from the 1950s onward, several attempts to image the BFM using electron microscopy were made (see Figure 3(A)).
Figure 2. A timeline of selected experimental and theoretical milestones in our understanding of the structure and function of the BFM, from the 1970s to the present.

First, the rough structure of the disk-like basal body was resolved [15,16]; however, the details of these disks remained unclear because they are surrounded by the bacterium’s cellular envelope. Later, in 1971, a series of papers described a way to purify the flagellar filament–hook–basal body complex (termed the intact flagella) [17–20]. These sketches of the fundamental features of the motor were enough to inspire the first quantitative modeling efforts; further structural information over the years has served to allow for more specific, detailed models, as well as to validate model predictions.

In 1994, researchers were able to isolate and characterize the basal body, together with the switching complex (responsible for changing the direction of motor rotation, e.g. from counterclockwise to clockwise, during chemotaxis; for more information on this topic, see [21]) [22]. With the development of cryoEM techniques, access to detailed knowledge of structure of the rotor increased drastically [23,24].

Obtaining similar information about the stator unit complex, however, has proven significantly more difficult. As this complex is fundamentally involved both in transmembrane ion passage and torque generation, atomic-level information of its structure is vital to our understanding of motor function. Investigations to this end have begun to be successful only recently, with the
structure of the \textit{Vibrio alginolyticus} stator unit complex, PomAB \cite{25}. The structure of the \textit{E. coli} stator unit complex remains elusive.

\subsection*{2.2. Tethered cell experiments (1974)}

The bacterial flagellum is approximately 20 nm in width – too thin to be visualized using conventional optical microscopy. Furthermore, this several micron-long filament moves rapidly, making characterizing motor rotation via the observation of flagellar motion prohibitively difficult. In 1973, Berg et al. showed strong evidence of rotation motion in bacterial flagella \cite{26}.

In 1974, Silverman and Simon overcame this obstacle by attaching cells to surfaces using antibodies \cite{27}. In this way, motor rotation could be characterized via rotation of the tethered cell (see Figure 3(B)). The load of the cell body is such that the motor rotates at only a few hertz, ideal for direct observation by eye. This experimental design has stood the test of time, remaining a practical and easy method to study the motion of the BFM. Sometimes, all that is needed to overcome an obstacle is a quick shot to change one’s Point of View.

\subsection*{2.3. Energy source (1977)}

ATP is known as the standard cellular energy ‘currency’. However, the flagellar motor is driven by a different energy source, the energy gained from the ion flux through the electrochemical potential across the bacterial cell membrane. This critical knowledge arose from two studies in 1977: it was shown that after starvation, swimming ability can be recovered through an artificial electrochemical potential in \textit{Streptococcus} (strain V4051) \cite{30} and, more specifically, by an artificial proton motive force (PMF) in \textit{Bacillus subtilis} \cite{31}. Shortly after, a study conclusively showed that the PMF, not ATP, was the driving force behind motor rotation \cite{32}.

The PMF is the sum of the electrical and chemical potential difference across a membrane (here, across the cellular envelope of the bacteria). It is given by:

\[ \text{PMF} = V_m - Z \Delta \text{pH}, \]

where \( V_m \) is the membrane potential, and \( Z = 2.303 \left( \frac{RT}{F} \right) = 2.303 \left( \frac{k_B}{e} \right) \), in which \( R \) is the gas constant, \( T \) is the absolute temperature, \( F \) is Faraday’s constant, \( k_B \) is Boltzmann’s constant and \( e \) is the electrical charge. The typical PMF for \textit{E. coli} grown at pH 7.0 is approximately \(-170 \) mV. The dependence of motor speed on the membrane potential \( V_m \) was shown to be linear (over the physiological range, i.e. up to \(-150 \) mV) in 1995 by an experiment wiring motors to an externally controlled voltage source \cite{33}.

We note that there exist bacteria that utilize the gradients of ions other than protons – for example, alkalophilic bacteria are driven by sodium ions. The
Figure 3. An overview of experimental landmarks. (A) Electron microscopy image of the *Salmonella* flagellar motor basal body. Figure adapted from [3]. (B) The tethered cell assay allowed for direct characterization of motor rotation. (C) The BFM is unique in harnessing the energy stored in the transmembrane ion gradient instead of ATP. Wild-type *E. coli* motors are driven by the proton motive force. The development of sodium-driven chimeric motors eased the difficulty of studying motor energetics, as bacteria are far more sensitive to changes in pH than in sodium concentration. (D) Resurrection of paralysed *mot* mutants demonstrated the existence of up to 11 independent torque-generating stator units. Figure adapted from [4]. (E) The bead assay, which involves observation of the rotation of small beads tethered to flagellar stubs, allowed for the characterization of motor dynamics across a wide range of external loads. (F) The torque–speed curve is the most fundamental dynamic measurement on the flagellar motor. The BFM’s curve shows two distinct regions: a constant torque plateau up until a ‘knee’, after which the torque rapidly falls until the zero-torque speed. Recently, it was discovered that the number of stator units a motor is able to maintain depends on external load: motors at high load are able to maintain many more stator units than motors at low loads. Figure adapted from [28]. (G) The development of the bead assay and sodium-driven motors allowed for the direct observation of motor stepping behaviour. Figure adapted from [29]. (H) Studies using fluorescently labelled stator units showed that the structure of the motor was dynamic, with components constantly turning over and being exchanged with a pool of proteins in the membrane.

The general term of Equation (1) for the *ion motive force* (IMF) is then:

\[
\text{IMF} = V_m - Z \Delta p(\text{Ion}) = V_m - Z \log_{10} \left( \frac{[\text{ion}]_e}{[\text{ion}]_i} \right),
\]

where \(p(\text{Ion})\) is the generalization of pH to any ion (i.e. the decimal cologarithm of the ion concentration, \(-\log_{10}[\text{ion}]\)), \([\text{ion}]_e\) and \([\text{ion}]_i\) are the extracellular and intracellular ion concentrations, respectively (see Figure 3(C)). Thus far, there has been only one ion flux measurement reporting that the BFM consumes roughly 1200 protons per revolution [34].
2.4. **Stator resurrection (1984)**

While the rotor of the BFM has been fairly deeply studied, the dynamics of the torque-generating stator units remain significantly more enigmatic. Each stator unit is a complex containing 4 MotA proteins, which contain the torque-generating domain that interacts with the rotor, and 2 MotB proteins, which contain the ion binding site and the domain that binds to the cell wall.

*Resurrection experiments*, which showed that the rotation speed of the motor can be recovered in a stepwise fashion in paralysed (stator-less) mutants of *E. coli* via induction using Mot proteins, provided the first insight into the dynamics of these complexes [35,36]. Resurrection experiments on tethered cells showed eight stepwise jumps in speed [37]. Later, the development of the bead assay (see Section 2.5) demonstrated that these experiments could be performed with various external loads [38]; in 2006, resurrection experiments using attached beads showed the existence of up to 11 independent stator units (see Figure 3(D)) [39].

When this experiment was performed at low loads, however, researchers observed only a single jump to the maximum observed motor speed [40]. This was interpreted to mean that, at near-zero external load, only one stator unit was needed to rotate the motor as fast as it could go [4]. This quickly led to a series of reworked theoretical models, all of which fit the constraint of a universal limiting speed. However, much like the report of a barrier to backwards rotation, this idea came under question several years after its conception, when it was found that motors at ultra-low load are able to maintain fewer stator units loads (∼1–2 vs. ∼8–11 near stall; see Section 2.8).

2.5. **Application of external torque (1993) and the bead assay (2000)**

The relationship between the motor torque and speed is one of the most fundamental dynamic features of the BFM. The first attempt to explore this relationship was a 1987 experiment that used metabolized cells of motile *Streptococcus* in different viscous environments [41]. However, as previously discussed, inferring the motor speed from observing flagellar motion is difficult and indirect. Furthermore, the estimated torque in these measurements also depended on several varying factors, such as the size of the cell body. Accordingly, it was measurements made using tethered cells that provided the first truly quantitative estimate of the torque–speed curve [42]. Further understanding the BFM’s torque–speed relationship was made possible by two experimental leaps: the development of (1) methods to apply external torque to the motor and (2) the bead assay.

These techniques proved particularly important to study this relationship in regimes that could not be reached using the limited range of loads allowed by tethered cell experiments. Experiments utilizing a rotating electric field (*electro-rotation*) reported that there was a barrier to backwards rotation of the motor;
that is, significantly more applied torque was required to rotate the motor slowly backwards than to rotate the motor slowly forwards or stall it [42]. The existence of such a barrier strongly hinted that the motor may operate using a Brownian ratchet mechanism, and several models were developed to this end. However, a few years later, optical trap experiments showed that this observed barrier was an artefact of the electrorotation method, and a flurry of revised, non-ratchet models soon followed [43].

But what about the other end of the universe? Tethered cell experiments left the low-load, high-speed regime of the motor totally unexplored. This was remedied by the discovery that beads of varying size could be attached to the flagellar stubs of adhered cell bodies, and used to observe motor rotation at a wide range of loads. The possibility of the bead assay was demonstrated decades earlier, but the yield was low [27].

However, during an investigation into the minimum size of functional bacterial flagellin, a hydrophobic flagellar mutant was discovered [44]; this ‘sticky’ filament was then utilized in tethered cell experiments, and eventually to the bead assay with polystyrene beads [38,42,45]. Using this assay, the BFM torque–speed curve was shown to have two distinct regimes: (1) a constant torque at low speeds up until a critical ‘knee’, after which (2) motor torque rapidly drops until the zero-load speed is reached (Figure 3(E)) [45].

Several further advancements in our understanding of this curve have been made in recent years: resurrection experiments using the bead assay reported torque–speed curves for motors with different stator unit numbers [38]. Currently, high-resolution data can be taken using a back-focal plane detection system for loads as low as 200 nm. To probe the ultra low-load region, smaller beads must be attached to the hook. To this end, nano-gold particle beads assay has been developed and via different detection methods [40,46], nearly complete curves (i.e. curves extending almost up until the zero-torque speed) have been measured for the BFM [47].

2.6. Chimeric motors (2003)

The proton gradient drives the E. coli flagellar motor. As noted in Equation (1), varying the PMF requires changing either the bacterium’s membrane voltage or the pH of the medium. Unfortunately, as robust as they may seem, even bacteria have their limits: measurements on the BFM made over a wide range of PMFs have been stymied because bacteria cannot survive under such conditions.

Noting that several marine bacterial species have sodium-driven flagellar motors, researchers created the first chimeric sodium-driven BFM in E. coli in 2003 (see Figure 3(C)). To harness the electrochemical energy of sodium ion, the chimeric stator complex contains PomA, V. alginolyticus’s analogue of MotA in E. coli; and PotB, a mash-up of PomB N-terminus and MotB periplasmic C-terminus [48]. Important electrostatic interactions between the stator and the rotor in E. coli were demonstrated to retain their role when PomA is part of the
chimeric PomA/PotB stator in *E. coli* [49,50]; interestingly, however, they are significantly less important for rotation in *V. alginolyticus* [51]. While able to reach higher motor speeds and torques than the wild-type proton-driven motor, the chimeric motor has been shown to be qualitatively similar to that of *E. coli* with regard to several properties, including the torque–speed relationship [52].

This swung the door into the study the flagellar motor wide open – the coupling of sodium ions is far weaker than protons in *E. coli*, allowing quite a bit of flexibility in the ability of researchers to manipulate the ion motive force [53,54]. This ability was soon exploited, and single-cell measurements of the two separate components of the ion motive force were reported [53,54]. Using the bead assay, researchers also reported the torque–speed curves of motors at various, known IMFs; these measurements were also used to calculate the number of ions required for a revolution of the motor [47].

### 2.7. Observation of motor steps (2005)

Structural information on the motor provided the first hints as to its modus operandi: the M- and C-rings were shown to have 24- to 26-fold and 34- to 36-fold symmetry, respectively [24]. This, combined with the existence of independent torque-generating stator units surrounding the rotor, pointed towards the existence of a rate-limiting process in the rotor–stator interaction, and accordingly, a discrete, ‘stepping’ rotation.

However, directly observing these steps has proven challenging for two reasons: (1) the BFM’s symmetry hints at far smaller individual steps than other motors that exhibit large linear or rotational steps; these small discrete events are difficult to distinguish in the inherently noisy background of the nanouniverse; (2) because motor behaviour is observed via rotation of an external load (either a tethered cell body or bead), motor steps are further smoothed out by the flagellar hook, a soft spring.

This leads to a catch-22 of sorts: to counter the smoothing effect of the hook, motor rotation should be observed in the low-load regime. However, the motor speed is high under these conditions, making small steps exceedingly difficult to parse. This is where the development of the chimeric flagellar motor becomes particularly useful: stepwise rotation is observable in the low-load regime when motors are slowed down by lowering the external SMF. The existence of 26 discrete steps per motor revolution was shown in de-energized chimeric *E. coli* motors (see Figure 3(G)) [29] and proton-driven de-energized *Salmonella* motors spinning both clockwise and counterclockwise [55]. Further experiments are needed to properly understand the energetic properties of the BFM at (ultra) low loads; recently, gold nanoparticles have made such exploration an exciting possibility [40,46].
2.8. Dynamic turnover of motor components (2006) and load-dependent assembly (2013)

One of the most surprising results in the study of the BFM has been the discovery that motor components are constantly being turned over. Using the bead assay and high-resolution measurements of motor speed, small stepwise speed changes were observed in fully resurrected motors [39]. Later, experiments using fluorescently labelled MotB showed that stators units do not remain engaged to the motor, but are dynamically exchanged with a large pool of ‘waiting’ complexes diffusing about in the cell membrane (see Figure 3(H)) [56].

The transition from liquid to surface living in bacteria (e.g. the formation of biofilms and swarming) is often triggered when bacteria ‘sense’ they are near a surface. This has long been thought of as an indication that bacteria have some mechanosensing ability. The recent discovery that motors at high loads are able to maintain a much higher number of engaged stator units than motors at low load suggest that this ability may arise from the flagellar motor [57,58], clearing a path towards an exciting new line of research to be explored.

While we have gained further insight into the mechanism behind stator dynamics, similar results have been discovered regarding the turnover of rotor components such as FliM [59,60] and FliN [61,62] in the C-ring. The dynamic turnover of rotor components, which serves to explain the symmetry variation observed in EM images, is believed to be related to switching in the BFM [62]. These emerging studies strongly suggest that adaptive remodelling is a common feature in the BFM, and likely in molecular motors in general.

3. Theoretical milestones

The ultimate question of how the flow of ions across the bacterial cell membrane is transduced into torque by the flagellar motor is a fundamental problem in molecular biophysics, requiring deep thought. Several models have been proposed over the years to tie together the various experimental measurements made on the motor into a coherent framework to describe the mechanochemical cycle of this machine (Table 1). In the following section, we describe how hypothetical mechanisms for the flagellar motor have evolved, alongside experimental breakthroughs, since its initial discovery as the driving force behind bacterial swimming.

3.1. Oosawa and Hayashi (1986)

One of the first models to provide sufficient detail to quantitatively reproduce experimental observations on the relationship between motor speed and rotary torque was that of Oosawa and Hayashi [63]. Electron microscopy study had found two rings, composed of several radially arranged proteins, at the base of the flagellar filament [17]. Studies on the motility of bacterial cells after starvation provided evidence that this motor was driven by the flow from protons from the
cell’s exterior into the cytoplasm, quantifying for the first time the relationship between the motor’s speed and electrochemical gradient across the membrane \[30,32\].

The authors’ ideas stemmed from these large experimental strides: they postulated a mechanism in which two concentric rings interact with each other to generate torque and rotate the motor. The authors supposed that the outer ring, the S-ring, was fixed to the cell membrane, while the inner ring, the M-ring, was attached to the base of the flagellum. Proteins along the periphery of S-ring were purported to alternate between two positions in order to shuttle protons across the membrane. The interactions of these molecules with analogous ‘outer’ and ‘inner’ tilted positions on the M-ring were implicated as the mechanism of torque generation in the motor; the broad minima of binding free energies due to the flexibility of the sites provided the source of the model’s argument that ion flux was *loosely coupled* to motor rotation (i.e. each ion passing does not necessarily confer a full motor step).

Oosawa and Hayashi’s paper was seminal in strongly arguing for the close interaction between theory and experiment, making it an appropriate starting point in our timeline of milestones in our theoretical understanding of the flagellar motor. Further, the authors emphasized that proper analysis and understanding of experimental data requires an underlying model, and that even a shortage of structural information should not discourage theorists from constructing quantitative, experimentally testable models. At the time their model was constructed, it was not even known which parts of the motor might be rotating and which parts might interact with protons.

### 3.2. Läuger (1988)

As dynamic and structural experiments revealed more and more about motor function, so increased the number and specificity of modelling efforts. Torque-generating stator units were shown to not be a ring attached to the membrane, but instead possibly independent elements embedded in the cytoplasmic membrane, peripheral to the rotor ring \[35,36\]. Läuger proposed and analysed possible

| Model(s)            | Class                 | Coupling | Rotor-stator interaction | Zero-torque speed | Citation |
|---------------------|-----------------------|----------|--------------------------|-------------------|----------|
| Oosawa and Hayashi  | Ion turnstile         | Loose    | Not specified            | Not specified     | [63]     |
| Läuger              | Conformational change | Tight    | Elastic cross-bridge     | Not specified     | [64]     |
| Meister et al.      | Ion turnstile         | Tight    | Elastic cross-bridge     | Not specified     | [34]     |
| Berry               | Ion turbine           | Loose    | Electrostatic            | Not specified     | [65]     |
| Elston and Oster    | Ion turbine           | Loose    | Elastic-electrostatic    | May increase with stator number | [66] |
| Xing et al.         | Conformational change | Tight    | Not specified            | Decreases with stator number | [67] |
| Meacci and Tu       | Conformational change | Tight    | Not specified            | Independent of stator number | [68] |
| Mora et al.         | Conformational change | Tight    | Steric                   | Independent of stator number | [69] |
| Mandadapu et al.    | Conformational change | Loose    | Steric                   | Increases with stator number | [70] |
kinetic mechanisms for motor rotation [64]. Two possible torque-generating interactions between the stator elements and the rotating M-ring were considered.

In Model I of [64], force is generated via the simultaneous interaction of a proton with elements on the stator and rotor as follows. Stator elements were thought to be affixed to the cell wall, each containing a ligand row perpendicular to the plane of the ring (Läuger correctly identified MotB as a likely part of the stator complex). However, the interaction between an ion and one of these positions is not sufficient to remove it from solution: an energetic landscape favourable for ion passage occurs only at the intersection point between these and analogous ligand rows along the circumference of the M-ring, tilted with respect to those on the stators. This constrains the motion of the ion to this point alone, resulting in rotation of the M-ring with every ion passage.

The second model proposed by Läuger (Model II) differs from Model I in that the proton interacts with the stator and rotor sequentially rather than simultaneously. Ion translocation induces a conformational change in the stator elements, driving the movement of the rotor. The mechanism put forward by Model II of [64] is qualitatively similar to that of Oosawa and Hayashi’s model, with the exception that it proposes that motor rotation is tightly coupled to ion flow. This property arises from the fact that the binding between the stator and rotor in both configurations (‘outer-‘ and ‘inner-facing’) is strong, in contrast to the broad minima seen in Oosawa and Hayashi’s model. This requires that the transition between them (and thus, the passage of an ion) is tightly coupled to the movement of the inner ring – that is, each ion passing leads to a full motor step of a fixed size.

3.3. Meister et al. (1989)

Meister et al. proposed another tightly coupled mechanism for torque generation in the motor. In this model, similar to those put forward by Läuger, stator elements are positioned along the periphery of the rotating M-ring [34]. Stators are equipped with channel complexes composed of two half-channels that span the membrane. Adjacent sites on the rotor align with these two half-channels, resulting in one site being in contact with the cytoplasm (the $i$-site) and the other with the extracellular medium (the $o$-site).

Motion of the stator relative to the M-ring is constrained as follows: the ends of the channel cannot move past an occupied site, while the centre of the channel cannot move past an empty site. Consider a situation in which both extra and intracellular sites are bound; then, the channel can only move towards the $i$-site. If thermal motion carries it one step in this direction, the proton in the $i$-site is dropped off into the cytoplasm and the other bound proton moves into the intracellular position, leaving/allowing a proton from the extracellular medium to move into the empty $o$-site. This motion exerts a force due to the elastic linkage in the channel complex, inducing a rotation in the M-ring.
This model capitalized on the development of the tethered cell assay, and the subsequent improved ability to characterize the motor’s torque–speed relationship in the intermediate range. Importantly, this model predicted that the torque required to drive the motor backwards would steeply increase, a claim which would be ‘proven’ and then, interestingly, later ‘disproven’ as various techniques to apply torque to tethered cells were developed and improved upon.

3.4. Berry (1993)

Improved structural and biophysical experiments on the flagellar motor provided much information into the structure and function of the motor’s stator. The stator was found to consist of up to eight independent membrane-embedded mot units peripheral to the rotary rings, each capable of applying approximately equal torque to the rotor in either CW or CCW direction [36,37].

Given this information, Berry proposed a model in which alternating lines of high and low electrical potential are created along the perimeter of the rotor ring by the arrangement of positive and negative charges [65]. These lines are tilted with respect to the proton-conducting channels in the stators, such that protons flowing through the stator exert a long-range electrostatic torque on the rotor charges, forcing motor rotation.

This ‘ion turbine’ model is structurally similar to Model II in [64], where protons are constrained at the intersection of two ‘half-channels’ on the rotor and stator. However, it does not force tight coupling, instead relying on energetic constraints to keep protons close to the negatively charged lines on the rotor. Furthermore, this model explicitly implicated an electrostatic force as driving both motor rotation and motor switching, contrary to previous models which required a major conformational change for switching between CW and CCW modes.

3.5. Elston and Oster (1997)

Many windows into the function of the flagellar motor opened up with the development of biophysical techniques to apply torque to tethered cells. One such technique, electrorotation, applied torque to cells by high-frequency rotating electric fields displayed an apparent barrier to backward rotation [71].

This result yielded support for models utilizing a thermal ratchet mechanism for motor rotation. One of these was another ‘ion turbine’ model proposed by Elston and Oster [66]; the authors presented this model in the context of a motor with stator elements that were either fixed or movable. In the fixed-stator case, Elston and Oster’s model is similar to Berry’s, purporting that motor rotation was driven by electrostatically driven torque.

However, the movable stator case removes the requirement of a continuous proton path between the extracellular surface and the cytoplasm; the authors point out that such a physical separation facilitates the operation of a proton turbine, as it lowers the probability of proton movement without motor rotation.
due to thermal oscillations alone. Additionally, while the movable stator case still implicated electrostatic forces generated by tilted lines of alternating high and low potential along the rotor’s periphery in torque generation, it additionally considered the contribution of elastic and steric forces caused by conformational changes in the stator.

Though the results of early electrorotation experiments brought about a series of models based on the fact that there was a steep barrier to backwards rotation, later experiments with optical tweezers showed that this was actually an artefact: in fact, the motor generated the same torque when rotated slowly forwards or backwards [43].

This result excitingly turned the field upside down and forced researchers to reconsider one of the fundamental features of most published models. Interestingly, the next ‘round’ of models took a cue from Elston and Oster’s movable-stator model: with evidence that there was no innate energy barrier to backwards rotation, a power stroke driven by conformational changes in the stator elements became a likely contender for the flagellar motor’s torque-generating mechanism.

### 3.6. Xing et al. (2006)

A deeper exploration of the relationship between motor speed and rotary torque became possible with the two experimental developments: electrorotation (described previously) and the bead assay. In the bead assay, rather than observing rotating cell bodies tethered to glass slides by their flagella, the rotation of polystyrene beads attached to flagellar stubs was analysed [38]. Because beads far smaller than cell bodies were able to be used, this assay allowed the measurement of motor dynamics over a far larger range of torques than in previous experiments.

Both techniques showed the same result: the flagellar motor’s torque did not simply vary linearly with speed as previously thought, but instead displayed two regimes. At a fixed pmf, the torque generated by the motor was approximately constant up to $\approx 170$ Hz at $23^\circ$. Past this speed, the torque drops rapidly and linearly to zero at $\approx 300$ Hz.

This new information into motor behaviour was incorporated by Xing et al. into a model with four physical ‘ingredients’: (1) load and motor are connected via a soft elastic linkage; (2) motor rotation is tightly coupled to ion flux; (3) motor rotation is driven by proton-driven conformational changes in the stator units; and (4) the proton channel in the stator is gated by rotor movement. The third assumption was supported by experimental evidence of such a change in the stator complex [72]; it is worth noting that most models after these experiments implicated a conformational change in stator structure to drive motor rotation. However, because of a lack of atomic-level structural information of the motor, the authors chose to keep the details of the stator–rotor interaction vague beyond these four constraints [67].
3.7. **Meacci and Tu (2009)**

Two (more) surprising discoveries about the function of the flagellar motor came in quick succession. First, due to increases in experimental resolution, the motor was shown to proceed by steps at low speeds [29]. Second, ‘resurrection’ experiments (described previously) seemed to show that motor speed at low loads was independent of stator number; that is, when the external load is near zero, one stator seemed to be able to rotate the motor as fast as it can go [40]. The idea that the zero-torque speed must be independent of stator number in particular led to the development of several new models.

Meacci and Tu proposed a kinetic model that explained this behaviour based on the assumption that the stepping rate of the stator elements was dependent on the torque exerted on the rotor, analogous to the Huxley model for myosin – that is, ‘negative’ torque between the rotor and a stator element (i.e. torque opposite the direction of motor rotation) increases the stator stepping rate [68,73]. This results in a zero-torque speed dependent only on the maximum stepping rate, but independent of the number of stator elements, as Yuan and Berg’s experiments predicted [40].

This kinetic model also intrinsically reproduced the motor’s observed stepping behaviour by the inclusion of two timescales in a single mechanochemical cycle: the waiting time $t_w$ depending on the chemical transition of the stator and the moving time $t_m$ determined by the mechanical rotation of the rotor ring. A future iteration of this model addressed the absence of a barrier to backwards rotation through the inclusion of a back-stepping probability [73].

3.8. **Mora et al. (2009)**

In step with Meacci and Tu, Mora et al. also capitalized on these new insights to propose a simple physical model for motor rotation [69,74]. Their model relied on two main assumptions: (1) all stator elements work in unison, applying torque to the motor simultaneously and additively and (2) this torque is imparted via a contact force between the stator and the rotor. Each stator complex was modelled as a set of protein springs that detach and reattach from successive sites along the rotor’s circumference with each ion passage. This simple physical model, in which the stator drives a ‘bumpy’ rotor through a viscous medium, fundamentally utilized the physical structure of the motor and was shown to be consistent with all the experimental data available at the time.

3.9. **Mandadapu et al. (2015)**

The most recent shock to the system came a few years later, when experiments by Lele et al. showed that the number of engaged stator elements in a motor was dependent on the external load [57]. This result brought into question several previous results, and, importantly, lifted the constraint regarding a stator-independent limiting speed: if motors near zero load likely only had one or two
stators, interpreting the results of low-load resurrection experiments became far more difficult.

This opened the door to new modelling efforts. The idea of revisiting the theory behind the flagellar motor’s fundamental torque-generation mechanism was made even more timely by measurements made on single-stator chimeric sodium-driven motors [47], which removed complications associated with interactions between individual stator elements. Mandadapu et al. [70] did just this, combining all the available experimental evidence to propose a mechanically specific model for torque generation.

This model combined elements from several recent models. Like Mora et al., they indicated a contact force between the stator elements and the rotor as the torque-generating mechanism [74]; however, they further proposed a more detailed mechanism for this steric interaction, involving a conformational change about a conserved essential proline residue in MotA (Figure 4). Like Meacci and Tu, their proposed mechanochemical cycle consisted of alternating ‘waiting’ and ‘moving’ times; simulations from this model for motors with a single-stator element reproduced torque–speed curves from chimeric single-stator motors [47].

Mandadapu et al. cast their model into the form of three Langevin equations, one each for the motion of the stator, rotor and external load (in the lab, either a tethered cell or a small bead attached to the flagellar hook). The dynamics of the angular positions of the stator loops $\phi_S(t)$ and the rotor $\theta_R(t)$ are represented as:

$$\zeta_S \frac{d\phi_S}{dt} = F_p \ell_p - \tau_{\text{reaction}} + \sqrt{2k_BT}\xi_{Sf_n}(t)$$

where:
- $\zeta_S$ is the viscous damping coefficient.
- $F_p \ell_p$ represents the torque from the proline hinge.
- $\tau_{\text{reaction}}$ is the reaction from the rotor.
- $\sqrt{2k_BT}\xi_{Sf_n}(t)$ represents thermal fluctuations.

**Figure 4.** Overview of the proposed torque-generating mechanism of Mandadapu et al. Cation binding induces a strain in the stator, which causes the loops to bend. Notes: This results in the first half of the power stroke (here, by Loop 1 of the stator), and sets up the second loop (here, Loop 3) to perform its half of the power stroke. Subsequently, the cations are released into the cytoplasm; this is possible because the proposed motion also has a vertical component – the loops lower themselves out of the membrane. This release then reverses the strain and causes the loops to restraighten. This results in the second half of the power stroke. This image depicts a two-dimensional projection of a three-dimensional motion: stator motion is not constrained to the plane of the page.
\[ \zeta_R \frac{d\theta_R}{dt} = \frac{\tau_{\text{contact}}}{\text{Torque from stator}} - \frac{\kappa(\theta_R - \theta_L)}{\text{Spring connection to load}} + \sqrt{2k_BT\zeta_R f_n(t)}, \]

where the final term in each equation is the stochastic Brownian force, in which \( k_BT \) is the Boltzmann constant multiplying temperature and \( f_n(t) \) is uncorrelated white noise.

The dynamics of the load \( \theta_L(t) \) are described by:

\[ \zeta_L \frac{d\theta_L}{dt} = \kappa(\theta_R - \theta_L) + \sqrt{2k_BT\zeta_L f_n(t)}. \]

The model assumes that stators apply no force (\( F_p = 0 \)) to the rotor between power strokes. This results in negligible applied (\( \tau_{\text{contact}} \)) and reaction torque (\( \tau_{\text{reaction}} \)) during the waiting times \( \tau_w \). Because the BFM lives at low Reynolds number, the rotor cannot ‘coast’, and so also exhibits no productive movement when the stator is disengaged between steps.

Because of the new freedom allotted to theoreticians due to Lele et al.’s experiments, Mandadapu et al.’s fundamental torque-generating model can explain several low-load scenarios when extended to model the dynamics of multi-stator motors. For example, if stator elements do indeed act in synchrony as Mora et al. suggested, then this model also predicts a zero-torque speed independent of stator number. However, if stator elements are considered independent Poisson steppers (which seems more likely given the lack of evidence that stators are able to ‘talk’ to each other and coordinate their stepping), Mandadapu et al.’s model suggests that the motor’s behaviour at low load is not all that different from that at high load: motor speed will increase as new stator elements are recruited [28]. Experiments clarifying the true nature of the motor’s zero-torque dependence on stator number will be crucial in determining if and how elements in multi-stator motors interact with each other.

**4. Conclusions and outlooks**

The BFM’s fundamental role in facilitating several biological processes has made it one of the most well-studied molecular machines. BFM is far more complex than any other molecular machines we have known. The history of research on the BFM is a prime example of how valuable the close interplay between theory and experiment can be.

In this review, we hope to have emphasized that ‘reality’ is often inaccurate: even questions long believed to be closed must be periodically critically examined when new information arises. Models must constantly be updated as new experimental evidence arises, and theorists must always be aware and take account of all available knowledge in order to be able to provide the momentum for future experiments.
We also hope to have emphasized that despite the rich history of BFM research, there remain many future open questions about the function of this dynamic nanomachine. Atomic knowledge of the stator unit structure is crucial to our ability to understand motor function and build more detailed, predictive models. Many experiments remain necessary to validate the recent model predictions of the motor’s fundamental mechanochemical cycle. Even the basic question of whether the BFM’s rotation is loosely or tightly coupled to ion flux has not yet been definitively answered. Furthermore, the recent discoveries reporting the dynamic nature of motor structure have opened up a whole new avenue of research on how the BFM has evolved to interact with and adapt to its environment.

So, for the new generation of biophysicists: ‘Don’t Panic’. There is still a lot to learn!

Disclosure statement

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