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Ion-driven rotary membrane motors: From structure to function

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Abstract

Ion-driven membrane motors, essential across all domains of life, convert a gradient of ions across a membrane into rotational energy, facilitating diverse biological processes including ATP synthesis, substrate transport, and bacterial locomotion. Herein, we highlight recent structural advances in the understanding of two classes of ion-driven membrane motors: rotary ATPases and 5:2 motors. The recent structure of the human F-type ATP synthase is emphasised along with the gained structural insight into clinically relevant mutations. Furthermore, we highlight the diverse roles of 5:2 motors and recent mechanistic understanding gained through the resolution of ions in the structure of a sodium-driven motor, combining insights into potential unifying mechanisms of ion selectivity and rotational torque generation in the context of their function as part of complex biological systems.

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Introduction

Nature has evolved a series of membrane-bound protein machines to harness the potential energy stored across the membrane. Among them are rotary transmembrane motors. While the cell exerts significant effort to maintain the concentration of ions inside the cell, and the resulting membrane potential, these miniature motors convert this stored chemical energy into mechanical, rotational energy, which, in turn, is utilised to perform a diverse range of biological functions.

To date, there are only two known classes of ion-driven membrane rotary motors: the rotary ATPases and 5:2 motors (Figure 1a) [1]. Rotary ATPases synthesise or hydrolyse adenosine triphosphate (ATP), utilising or maintaining the membrane potential, respectively. 5:2 motors are named for the stoichiometries of their components: they consist of a cyclic pentameric ring of one protein outer encasing the dimeric coiled-coil domain of the other. The most well-studied of these are motility proteins A and B which form the complex $MotA_5B_2$ and drive the rotation of the bacterial flagellum.

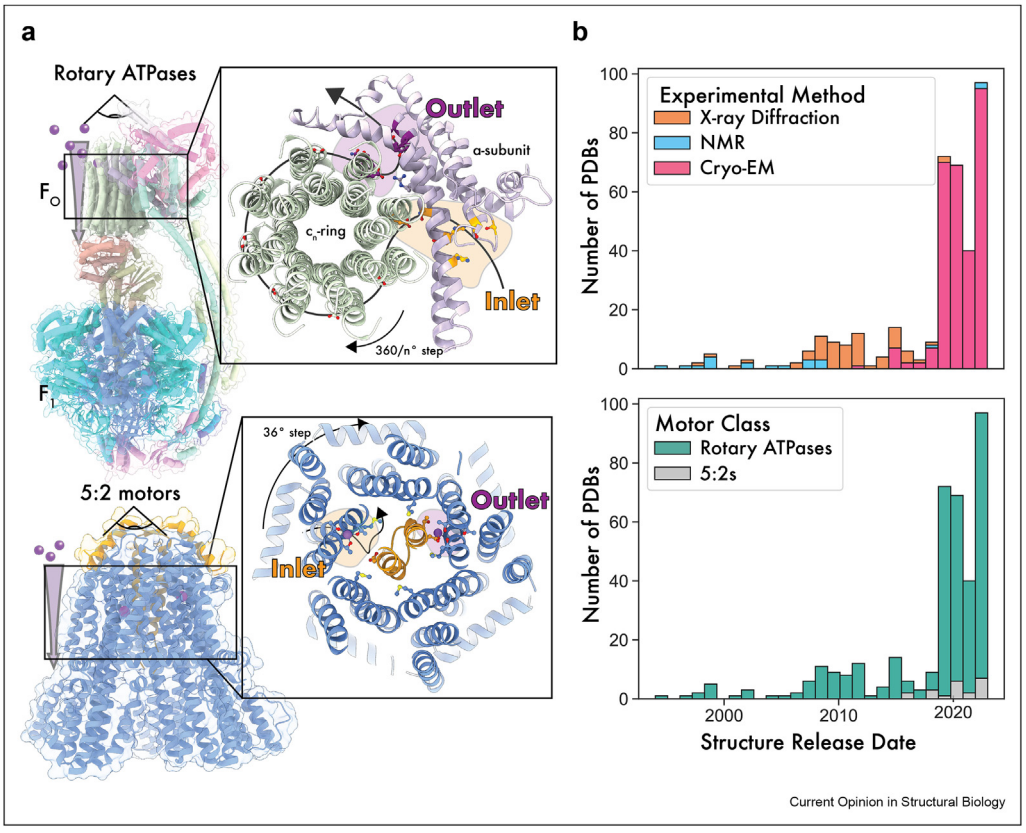
The resolution revolution in single-particle cryogenic electron microscopy (cryo-EM) [2,3] has led to a surge in the number of complete atomic structures of membrane motors, of these PDB entries, rotary ATPases account for 94% of membrane motor structures (Figure 1b). Since fewer structures of 5:2 motors have been determined, arguably, less has been understood about their mechanism of action.

Herein, we highlight the recent advances in the insight of ion-driven membrane-protein motors. These results give us a new understanding of the generation of torque from the IMF and ion selectivity in sodium and proton-driven variants. This review will only focus on the structural advances, for recent insights into their evolution and phylogeny please see work by Mahendrarajah and coworkers [4] and Nandel and coworkers [5] for ATP-synthases and 5:2 motors.

Rotary ATPases: From ion-driven energy factories to ATP-driven ion transporters

Rotary ATPases are ubiquitous across all domains of life and convert IMF into rotational torque, subsequently into stored chemical energy in ATP, which is then used in a multitude of processes throughout nature. Within rotary ATPases, there are several subtypes: the F-type and A-type use the ion-motive force (IMF) to synthesise ATP, whereas the V-type, which is found in eukaryotic intracellular membrane compartments, uses ATP as a

Figure 1



An overview of the structures of ion-driven membrane motors. (a) Overview of the structures of an example ATP synthase (top) and two 5:2 motors (bottom) each shown side on as surfaces coloured by chain and then with a slice through the membrane portion shown as cartoon ribbons. The F_O and F_1 domains of the ATP synthase are labelled. The rotational direction of each motor domain is also depicted by an arrow, structures are shown with the cytoplasmic side down. The c-ring is labelled with "n" subunits, and the rotation step is 360°/n, in this case, n = 8 and the step size is 45°.**(b)** PDB submissions by year broken down by experimental method (top) and the class of ion-driven membrane motor (bottom).

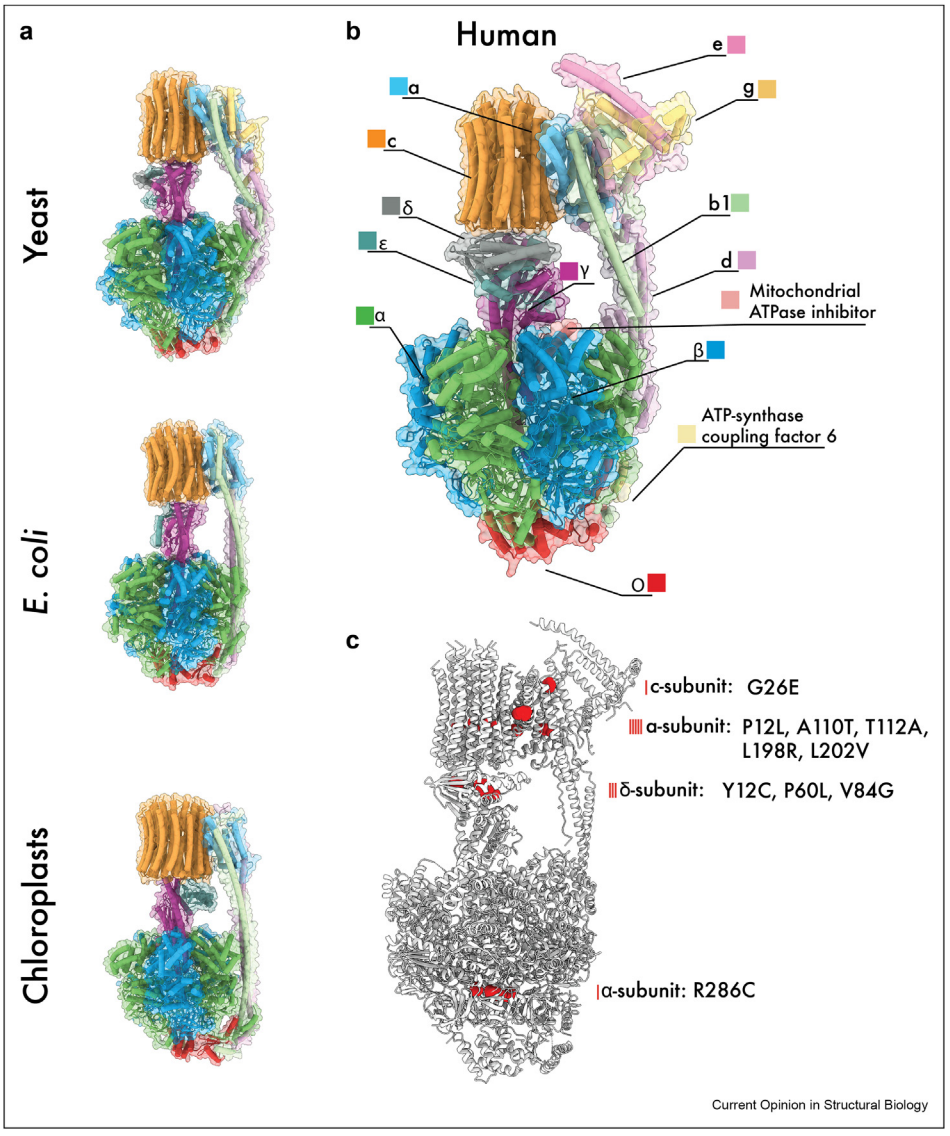
fuel source to transport ions across a membrane, against the chemical gradient. The F-type ATP-synthase is found in bacteria, mitochondria, and chloroplasts while the A-type is found in the plasma membrane of archaea and some eubacteria.

F, A, and V-types vary slightly in their overall architectures, but all contain two key domains X_O and X_1 (where X is the type). The F_1 domain is comprised of a hetero-hexamer of α and β subunits, the rotation of this machine has been directly observed [6]. ATP is synthesised in the F_1 domain powered by the F_O domain. The structures of the ion-translocation pathways in the F_O domain — between the a and c-subunits — are highly conserved [7–9]. The c-subunit makes a homo-oligomer with cyclic symmetry called the c-ring, containing between 8 and 17 subunits, depending on the species [10]. Moreover, studies have shown that some species vary this stoichiometry to adapt to environmental challenges [11]. The first structure of the F_1 domain of an ATP-synthase was published in 1994 [12] and of the F_O in 1999 [13]. However, until cryo-EM's coming-of-age, no structure of an entire membrane motor had been solved, rather crystal structures of segments were determined, and the complex pieced together [14–16].

The mechanism of ion-translocation that drives the rotation of the F_O domain was theorised [17] before multiple structural and molecular dynamics [18–20] studies confirmed, and built upon those theories. The first complete structure of an ATP synthase with a high enough resolution to resolve the residues involved in ion-translocation was by Hahn and coworkers [21] of the chloroplast ATP synthase followed shortly by Guo and coworkers [22] from bacteria. Now, structures of complete ATP-synthases have been solved across all domains of life (Figure 2a).

From these studies, the mechanism of proton-translocation in ATP synthases can be generalised: briefly, the inlet is lined with several conserved polar residues of the a-subunit, which provide an entrance for

Figure 2



F-type ATP synthases. Variations, mutations, and conservation. (a) Surface representation of ATP-synthase structures from (from top to bottom), yeast (PDB ID: 6C6P) [79], *E. coli* (PDB ID: 8DBP) [80], and chloroplasts (PDB ID: 6FKF) [21]. **(b)** The structure of the human F-type ATP synthase reported by Lai and coworkers [33] (PDB ID: 8H9S). **(c)** Clinically relevant mutations are highlighted in red and side chains are shown as spheres on the structure.

water to transport an ion to a conserved glutamate (or aspartate in *Escherichia coli.*) of the c-ring (shown in Figure 1a) [23]. It has been shown that the environment around this residue dictates the ion selectivity of the rotor [24,25]: sodium-driven c-rings have a pentavalent coordination to select for sodium whereas proton-driven rotors only need the protonatable sidechain and at least one hydrogen-bond donor to stabilise the interaction. A conserved arginine of the a-subunit stops the glutamate from rotating directly to the outlet and prevents the backflow of ions. Instead, the c-ring rotates clockwise (as viewed from the periplasm) opening another site for protonation. Once the glutamate rotates to the outlet in the a-subunit, again lined with several conserved polar residues, the ion is released into the cytoplasm. These residues change for ATP-driven ion pumps, contacts alter the pKas of the nearby residues tuning them for ion import or export across the membrane [26]. The rotation generated by the a and c units is then transferred up through the central stalk (subunits γ, δ, and ε) to power ATP synthesis in the α and β subunits, which are held in place by the peripheral stalk (subunits b or b and d) to prevent rotation without synthesis [27].

In mitochondria, ATP-synthases not only perform this vital role of generating stored chemical energy, but they also have an important role in shaping the membrane as multiple copies of ATP-synthases interact through

membrane-embedded subunits and induce membrane curvature away from the F_1 head domains, influencing the structure of the mitochondria [28,29]. Cryo-EM and cryogenic electron tomography (cryo-ET) have enabled researchers to visualise these structures in multiple oligomeric states in their native environments. For example, structures have been determined of monomers, dimers, tetramers [30], and hexamers [31] of F-type ATP-synthases. Furthermore, it has been suggested that the curvature they induce can modulate their activity [32].

Recently, Lai and co-workers published the structure of human ATP-synthase in four rotational states and placed these structures, and their function, in the context of clinical mutations related to diseases (Figure 2b) [33]. Three of these highlighted mutations occur at the interface between the a-ring and c-ring, or inside the c-ring itself, indicating that these mutations may interfere with the ion-translocation mechanism and therefore interfere with ATP synthesis (Figure 2c).

Mutations to ATP-synthases are of particular importance as they are linked to a wide range of diseases. Genetic mutations affecting the a and c-subunits have been linked to diseases and syndromes affecting neurological health [34], and conditions such as myalgic encephalomyelitis/chronic fatigue syndrome that have increased since the novel coronavirus pandemic, which has also been linked to deficient ATP production [35]. Furthermore, mutations in the F_O rotor are found in many cases of breast cancer [36]. With the wealth of structural information we now have, the challenge becomes how to use it to develop new treatments [37].

5:2 motors perform a variety of functions

5:2 motors are named after the stoichiometry observed in the complexes. Other ratios have been observed in other complexes, but these are likely non-functional assemblies [1]. Unlike ATP-synthases, 5:2 motors are not known to store chemical energy, but rather use rotational torque to power the import or export of substrates, or the motion of the cell. They are mostly found in prokaryotes because — for their function — the dimer of the 5:2 motor must be anchored either to the peptidoglycan (PG) layer (e.g., MotB) or cytoskeleton (e.g., GldL) (Figure 3).

The miniature motors that move bacteria: $MotA_5B_2$, $GldL_5M_2$, and AlgRQS

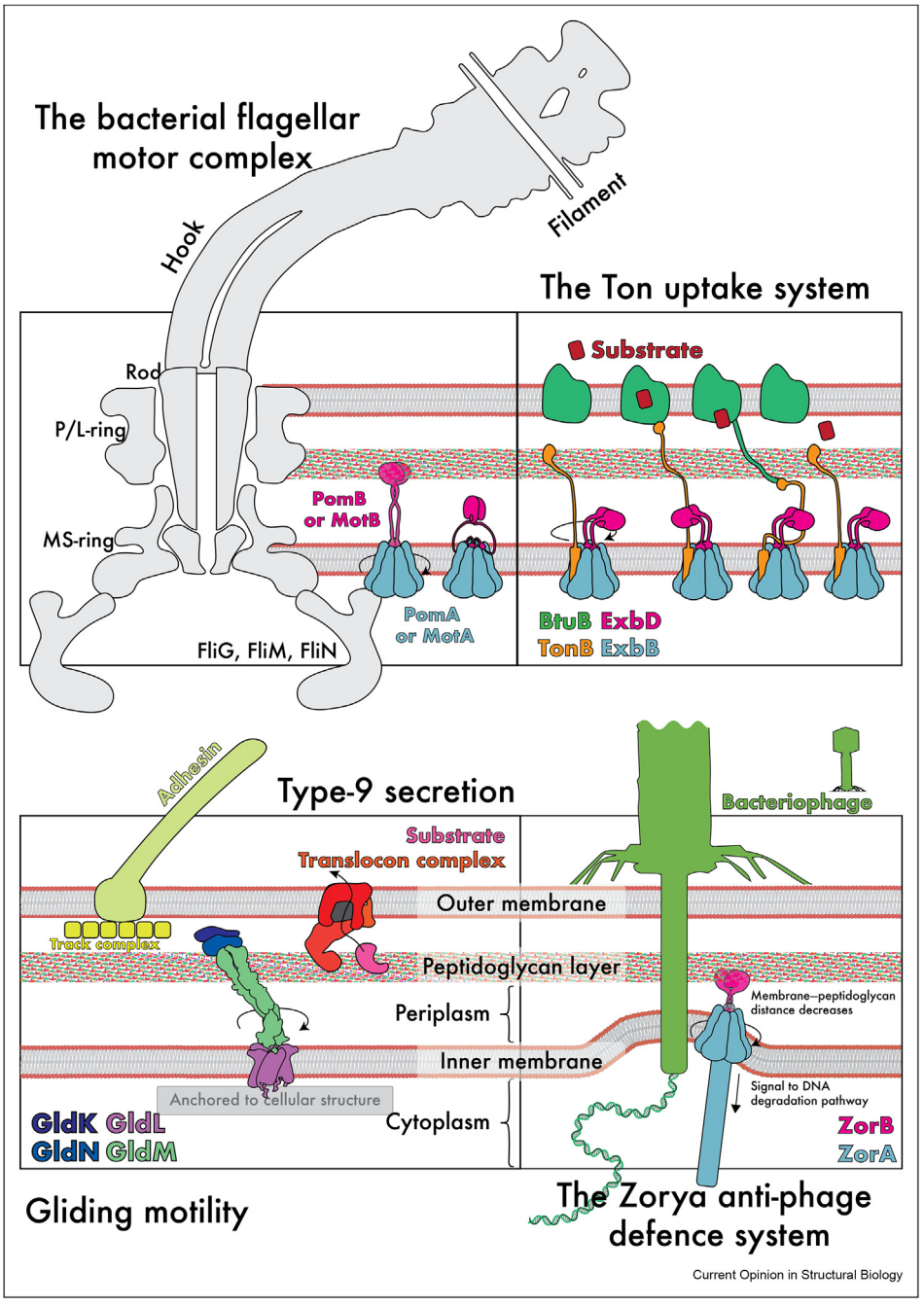
Bacteria use the chemical energy potential across the membrane to power their movement. This provides them with an evolutionary advantage to move towards nutrients and away from hostile environments, also known as chemotaxis. Many distinct mechanisms have evolved, It is worth mentioning the known examples of ATP-driven motility: one, via type IV pili through conformational changes in the cytoplasmic ATPases PilB and PilT [38]; two, a postulated motor with homology to the F_1 ATP-synthase domain in *Mycoplasma mobile* [39,40]; and three, archaea drive the rotation of their archaellum through an AAA+ ATPase, ArlI [41,42]. However, all known ion-driven motility relies on 5:2 motors [43].

The best-known of these is the bacterial flagellar motor complex (BFM), comprised of a rotor ring and a ring of stator units — the 5:2 motors — powering the flagellar rotation. In its inactive state, plug motifs from the dimer (e.g., $MotB_2$) inhibit the complex, preventing the leakage of ions from dysregulating the membrane potential [44,45]. The complex lies in the inner membrane (IM) and when it encounters the BFM, it activates: the plugs detach, and the peptidoglycan (PG) binding domains are able to dimerise, bind peptidoglycan, and anchor the stator unit. Now, ions permeating through the stator units drive the pentamer (e.g., MotA) to rotate around the dimer and the pentamer transfers this generated torque to the rotor of the BFM through its interactions with FliG [46—48].

It has been proposed that rotation occurs in 36° steps [49,50] in a turnstile mechanism [51] with MotB anchoring relative to MotA and performing a power stroke. One MotB is bound through its conserved aspartate residue and a bound ion MotA [52,53]. The other conserved aspartate is not bound to an ion, blocking the rotation of the non-polar surface of MotA along this negative charge. However, when the aspartate picks up an ion from the periplasm, rotation can occur. The first MotB releases an ion to the cytoplasm. The second MotB now takes the role of the first MotB and vice versa and the cycle can start again, for a predicted total of 10 ions transported per complete rotation of MotA around MotB [1].

Bacteria have evolved other methods of motility that do not require the BFM. Gliding bacteria secrete proteins called adhesins that attach to the surface they are moving on. The adhesins then move across the surface of the cell, pulling the cell along [43,54]. The gliding machinery and the type-9 secretion system export machinery are also powered by a 5:2 motor: GldLM or PorLM [55,56]. The structure of this 5:2 motor varies considerably from the others mentioned so far: there are only two short transmembrane helices of GldL/PorL compared to four in MotA. These transmembrane helices also have a higher tilt angle [57] with respect to the membrane. The central dimeric coiled-coil axle in GldM/PorM, is structurally conserved, but the sequences differ as there is no homologous aspartate residue for ion translocation. The mechanism by which the rotation powers the gliding is less well understood, but several key protonatable residues have been identified as important for motility [55]. It is believed that the pentamer (e.g., $GldL_5$) is stationary, anchored in the

Figure 3



The diverse functions of 5:2 motors. An overview of: the BFM complex (top left); the Ton uptake system outlining the mechanism described by Ratliff and coworkers (top right) [62]; The gliding motility system and type-9 secretion system powered by GldLM (bottom left) [55,81]; the Zorya anti-phage defence system (bottom right) [63,64]. All systems are shown as cartoons highlighting the action and rotation of the 5:2 motor within the context of the outer membrane, peptidoglycan layer, and inner membrane.

cytoplasm, while the dimer (e.g., $GldM_2$) rotates, also with a 36° step [55], pulling the bacteria along through adhesin filaments via the track complex.

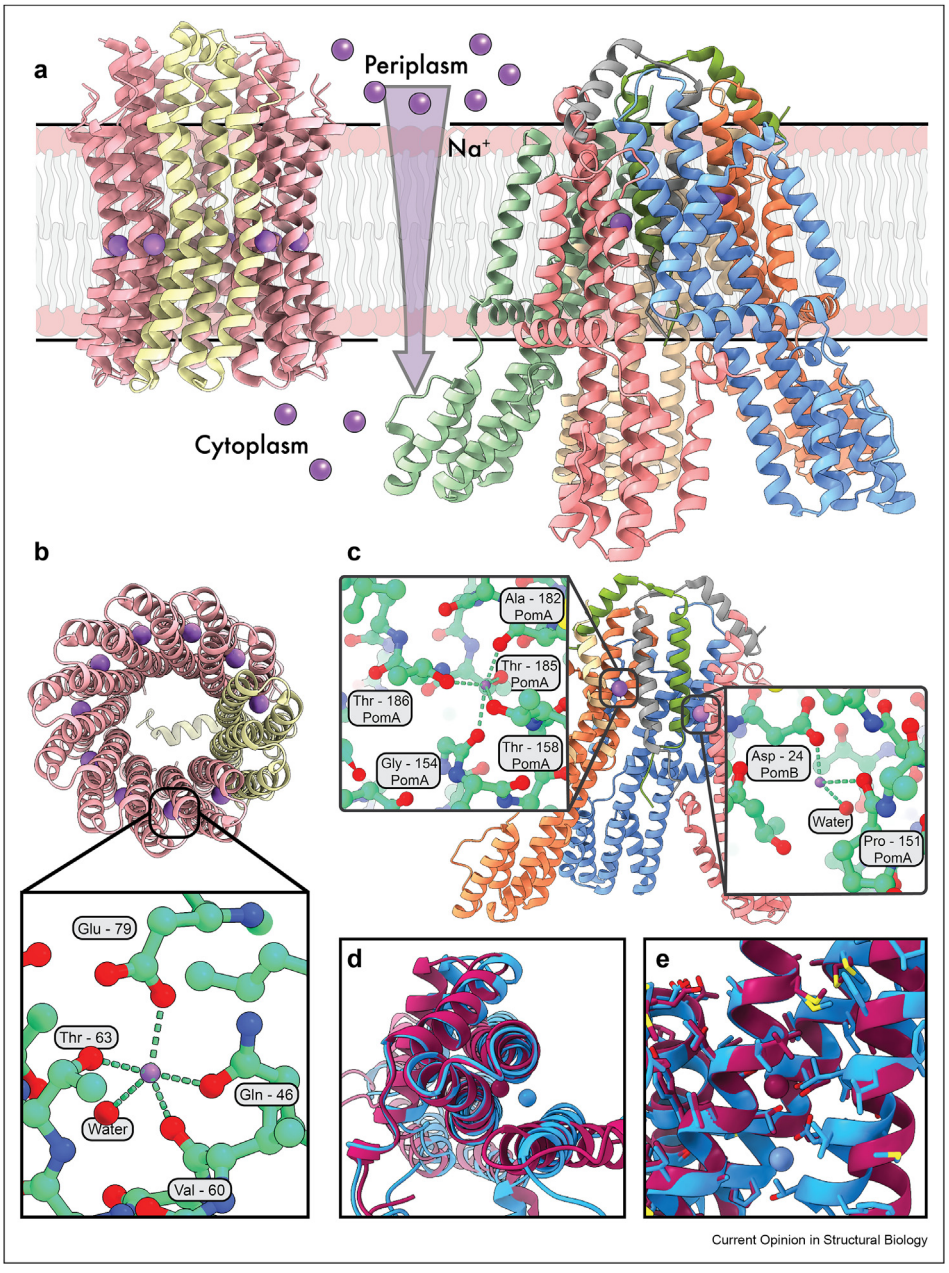
AglRQS is another predicted motor involved in an independent gliding mechanism. It is not closely sequence-related to Gld/PorLM [58]. The mechanisms are believed to differ considerably too, where GldLM remains stationary with respect to the cell, AglRQS moves along a track [59]. This system is less well understood, and no structural information exists for it. However, AglRQS is homologous to the MotAB/ExbBD

complex (AglR is homologous to ExbB/MotA, while AglQ and AglS are homologous to ExbD/MotB) [60], and not to Gld/PorLM, despite the shared gliding motility.

Hook, line, and sinker: The Ton complex and $ExbB_5D_2$

The Ton complex is powered by the ExbBD 5:2 motor [61]. The TonB links the inner-membrane bound motor to an outer-membrane TonB-dependant transporter (TBDT). The current best hypothesis for the mode of action is that as the complex rotates $ExbD_2$, bound to the periplasmic linker of TonB [62], winds the protein around the $ExbB_5$ motor, creating a downward force on TBDT, causing a conformational change, leading to the import of the exogenous substrate. It is not clear whether the pentamer or the dimer is stationary through the mechanism of action.

Figure 4



Mechanisms of ion selectivity in sodium-driven ATP-Synthases and 5:2 motors. (a) Side-on views of a sodium-driven ATP-synthase (left, PDB ID: 4BEM [66]) and 5:2 motor *Va*PomAB (right, PDB ID: 8BRD [65]). **(b)** Top-down view of the a-c ring complex of the sodium-driven ATP-synthase with an inlay zooming in on the sodium-coordinating residues. **(c)** Side-on slice through of the structure of PomAB showing the two resolved sodium sites with inlays zooming in on the respective sodium-coordinating residues. Structural overlay of the PomAB subunits from each sodium binding site (1: blue; 2: plum) shown from the top **(d)** and side **(e)** views.

Rotation resists phages: The Zorya defence system, ZorAB

In 2018, Doron and coworkers published the discovery of the Zorya phage defence system, which contains a membrane-embedded module that displays sequence-similarity to the MotAB complexes [63]. It is now uncovered that ZorAB has a MotAB-like rotary motor domain with a long, intracellular, helical tail, which shows a certain similarity to the cytoplasmic domain of prokaryotic chemoreceptor proteins. It is hypothesised that phage infection is detected by the peptidoglycan binding domains of ZorB and this signal is transduced down the tail through the rotation of ZorA, which recruits and activates DNA degrading enzymes to prevent phage replication [64].

Ion channel selectivity

For both ATP synthase and 5:2 rotary motor classes, structures of sodium and proton-dependent variants have been determined. This has enabled insights into how ion selectivity is achieved. Indeed, the mechanism of ion selectivity for ATP-synthases has been studied in detail [24].

The recently determined structure of the plugged sodium-driven stator unit PomAB from *Vibrio alginolyticus* at high resolution (down to ∼2 Å locally), reveals sodium ions in two positions in the cryo-EM reconstruction [65]. In this paper, it is demonstrated that the ion selectivity is dictated by the specific arrangement of five coordinating atoms from the protein and a highly coordinated water molecule, which would result in less energetically favourable coordination when either a proton, hydronium ion, or other cation is placed in the cavity.

Structures have also been determined for sodium-driven ATP-synthases [66], which allows for the comparison of how they might achieve ion selectivity (Figure 4a). In the chosen example of a sodium-driven a-c subunit complex, ten equivalent sodium ions are observed (Figure 4b). Each of these sites involved a penta-coordinated sodium in a trigonal bipyramidal geometry. In the structure of *Va*PomAB two sodium ions were observed in inequivalent positions (Figure 4c). The sodium will travel from the periplasm to the cytoplasm through sites 1 and 2 (Figure 4c–e). Site 1, where the ion enters the motor, has a similar penta-coordinated sodium ligated by 5 residues from PomA, whereas site 2 – where the ion is closer to the cytoplasm – has only 3 observed ligands.

The majority of sodium observed in proteins is in hexa-coordinated sites [67], where it would be most stable. The presence of the penta-coordinated sites implies the necessity for the number of ligands in specifying the ion while ensuring it is not too stable to impede the rotation of the motor. Further, the second site of PomA with only 3 ligands implies that the sodium is more weakly bound and therefore more likely to be released into the cytoplasm, preparing the stator unit for another turn. Importantly, as this structure of PomAB is in the plugged, inactive state, some of these interactions might also be different upon unplugging.

Conclusions

Nature harnesses the chemical potential across the membrane to perform an array of different functions by using a limited set of transmembrane rotary motors. Cryo-EM has made atomic structures of such membrane complexes more achievable; all complete structures of membrane motors have been determined by this method. The resolution revolution has improved and enabled the resolution of ions within these motors, giving key insights into the ion permeation pathways, and therefore, the mechanism of ion-translocation coupled torque generation. In recent years, tremendous advances have been made especially on rotary ATPases and their rotational mechanism. Creative biophysical experiments have also allowed for direct observation of the rotation of the ATP synthase [6] and bacterial flagellar motor [68] complexes. It is hoped that structural and functional insight into 5:2 motors will reach a similar level of maturity.

AI structural prediction methods have further revolutionised structural biology [69], and publicly available databases of predictions are now easily searchable [70]. With enough memory, predictions of full 5:2 motor complexes are easily accessible and can provide meaningful results [71]. However, the predictions of these complexes are almost always perfectly symmetric, unlike the distortions observed in the structure of PomAB. Predicted models often contain errors at domain, backbone, and sidechain levels [72], potentially combining these models with energetic functions allows for the creation of near-native models. However, there remains a gap that needs to be addressed and there is no replacement for the understanding gained through experimental structural methods. Furthermore, given the dynamic nature of these complexes, they inherently need to be modelled in different states, something that is currently very difficult using AlphaFold and related software [73–76]. Lastly, the inherent interaction with ions, the importance of a membrane gradient, and their embedding in the lipid bilayer all complicate prediction of structural mechanisms for membrane-bound rotary motors.

Furthermore, protein designers have set out to make programmable nanomachines, assemblies that can respond to stimuli and create motion, similar to motors found in nature. Already, Courbet, Baker and coworkers published the design of protein axle rotors [77]. Moreover, there is a growing toolkit of design software and building blocks that could be assembled [78], and its

rotation is powered by engineering natural or designed membrane motors. Based on this, and our advancing mechanistic understanding of ion-driven membrane motors, it could be possible to engineer native motors or design new motors to address new challenges across medicine and biotechnology.

It is clear that much still needs to be discovered about rotary motors, and will keep structural biologists, biophysicists, and computational biologists engaged for years to come.

CRediT authorship contribution statement

Freddie J.O. Martin: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Mònica Santiveri:** Visualization, Writing – original draft, Writing – review & editing. **Haidai Hu:** Visualization, Writing – original draft, Writing – review & editing. **Nicholas M.I. Taylor:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of Generative AI and AI-assisted technologies in the writing process

None.

Declaration of competing interest

The authors declare that they have no known competing financial interests, intellectual property, or personal relationships that could have influenced the work reported in this paper.

Data availability

Data will be made available on request.

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- • of special interest
- •• of outstanding interest

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