Do Ion Channels Spin?

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Ionic current flowing through a membrane pore with a helical architecture may impart considerable torque to the pore structure itself. If the channel protein is free to rotate, it will spin at significant speeds. Order of magnitude estimates of possible rotation rates are presented, as well as a few arguments why such motion could improve ion transport.

The interior of a living cell is maintained at a different electrical potential than the exterior, by differences in the concentration of various ion species [1]. Changes in this voltage, during action potentials for example, occur via ion flows through specialized pores in the cell membrane. The more rapid the ionic flows through the pores, and the more quickly they can be gated, the more efficient the overall system can be.

Recent advances in imaging pore proteins at the nanoscale have shown that pores are not just simple holes through the membrane, but are sculpted structures, particularly on the intracellular side of the membrane. For example, the elegant images of Sokolova et al [2] of the potassium Shaker channel show a "hanging basket" structure decorating the interior side of the membrane (Fig. 1). Recent images of sodium channel proteins by Sato and colleagues [3] and Payandeh et al [4] again display not just a simple hole, but an intricate series of tubes, suggesting a nanofluidic device (Fig. 2). In each of these examples there are lateral tubes, lying parallel to the membrane surface.

What are the functions of these shapes? As an evolved form, they need not have any single "function". But one can still speculate as to how a particular shape might facilitate the task of gating a stream of ions from one side of a membrane to the other. We will suggest that as part of this task, some membrane pore proteins may be in rapid rotary motion. By simple angular momentum arguments, if ions and water molecules follow any sort of spiral path through the membrane pore, rotary motion of the pore protein itself is not only possible, but likely. Further, ions driven by an electrical field along a helical path can generate considerable torque acting on the structure as a whole.

The motif of a spinning biochemical rotor is by now familiar. Besides bacterial flagella, we have the example of ATP synthase, a catalyst which operates a rotating motor on a molecular scale [5]. So if it can be demonstrated that ionic pores rotate during conduction, we simply have another example of a known architecture, albeit one that perhaps at least temporarily takes over the title of "smallest biological motor".

I. THREE ARCHITECTURES

A variety of pore protein structures have been visualized, at increasing resolution. They typically are composed of subunits, assembled with a twist, a definite handedness. Here are a few examples from recent literature, interpreted with a view towards possible pore rotation.

A. The "lawn sprinkler"

Payande et al [4] present remarkable images of a voltage-gated sodium channel, produced by crystalizing the pore membrane protein. Figure 2 sketches what they observed. Four subunits assemble into a structure shaped like a top, with an embedded pinwheel pattern of channels connecting the central pore to the outside. Their work, in accordance with conventional understanding, assumes that the central pore passes all the way through the protein, and that this is the path that the sodium ions take. They note though that the supposed "activation gate" at the bottom of the protein is closed, and that "it is surprising" to find a closed gate under the conditions in which the protein crystal was formed. They suggest that the lateral channels somehow close up when the channel is being gated.

However let's assume for the sake of argument that the flow in fact travels the paths revealed in the new images, that is, down the center from the top, then out the sides through the bent chanels. This leads to a rather different conception of the flow pattern. The sodium ions exit the pore protein laterally, and then proceed downward to the cytoplasm along the *outside* of the protein.

As indicated in Fig. 3, this fluid path will add a large helical component to the path. First, there is the pinwheel structure of the lateral channels, as shown in the left panel of the figure. This causes a clockwise turn in the flow. Then, there are the helical grooves along the outside of the protein, which act to impel a further rotation of the flow, again in the clockwise direction, as can be seen in the stereogram in the right side of the figure. As will be discussed later, both these effects result in a reaction torque of the rotor in the opposite counterclockwise direction.

Note that there is an additional sharp right-hand and downward curve at the outer ends of the lateral channels. The explanation for the existence of these channels suggested in Ref [4] is that they provide access to the central pore for lipid soluble drugs. But the form of these passageways seems not suited for direct access to the center, and argues instead for a much more dynamical function.

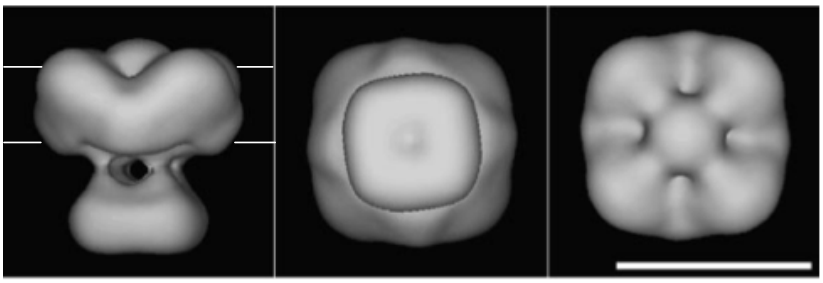

FIG. 1: Image of the "Shaker" potassium channel from Sokolova et al [2]. From left, side view (with indication of membrane location), bottom view, top view. Scale bar is 100Å. Note the four windows at the bottom, and the four exit pores at the top. There is a *twist* in the fluid path between bottom and top.

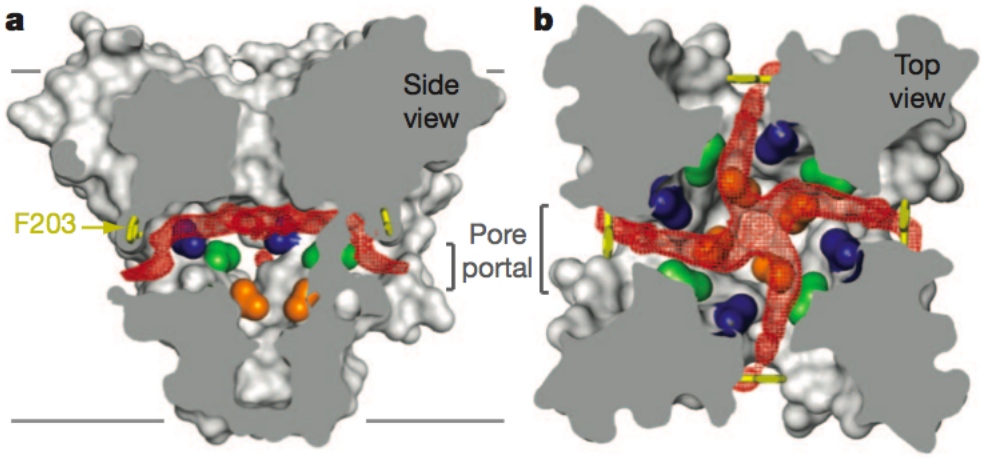

FIG. 2: A voltage-controlled sodium channel, as imaged by Payande et al [4]. The central tube of the protein is connected to the outside by pinwheel-shaped channels (colored red).

Figure 4 sketches a side view of this protein, which emphasizes the prominent spiral vanes running along the outside of this structure, at about a 45 degree angle, and the pinwheel shape of the interior channel. On the face of it, at least to a naive observer, this looks a rotor, which might act as some sort of pump or turbine.

Adding details to a speculation increases the probability of being wrong, nevertheless consider the cartoon of sodium channel ion gating drawn in Fig. 5. The protein as a whole lifts up, and then spins, allowing ion passage on the outside. There is perhaps an attractive simplicity to this mechanism. For example, when the channel is open, not all ions need pass through the "selectivity filter" located in the center of the pore, thus increasing overall flow. In addition, it might help explain the movement of observed "gating charges" which precede the flow, as well as the action of certain lipid-soluble toxins which only act when the channel is open [1].

B. The "four-by-four"

The images in Sokolova et al [2] of the "Shaker" potassium channel, produced using cryo-electron microscopy, again did not reveal a simple hole through the membrane, far from it. Instead, as sketched in Fig. 1, ions apparently enter the pore laterally through four windows in a large "hanging basket" structure which extends into the cytoplasm, then exit through four holes at the outside of the membrane. Now if the two sets of four holes were at the same relative angle, we would expect no net torque due to the flow, by symmetry. But in fact a net twist was observed, which breaks the symmetry. It appears that the fluid flow undergoes a 45 degree turn as it passes through the top part of the structure. A 45 degree twist is also reported between upper and lower orifices in a sodium channel imaged by by Sato et al [3], although the geometry of the plumbing of this channel is again not yet clearly resolved.

The "hanging basket" or "gondola" seems to be a recurring theme in the flood of striking images that have been appearing. In an intriguing recent paper, Clarke et al [6] explicitly address the torsion between top and

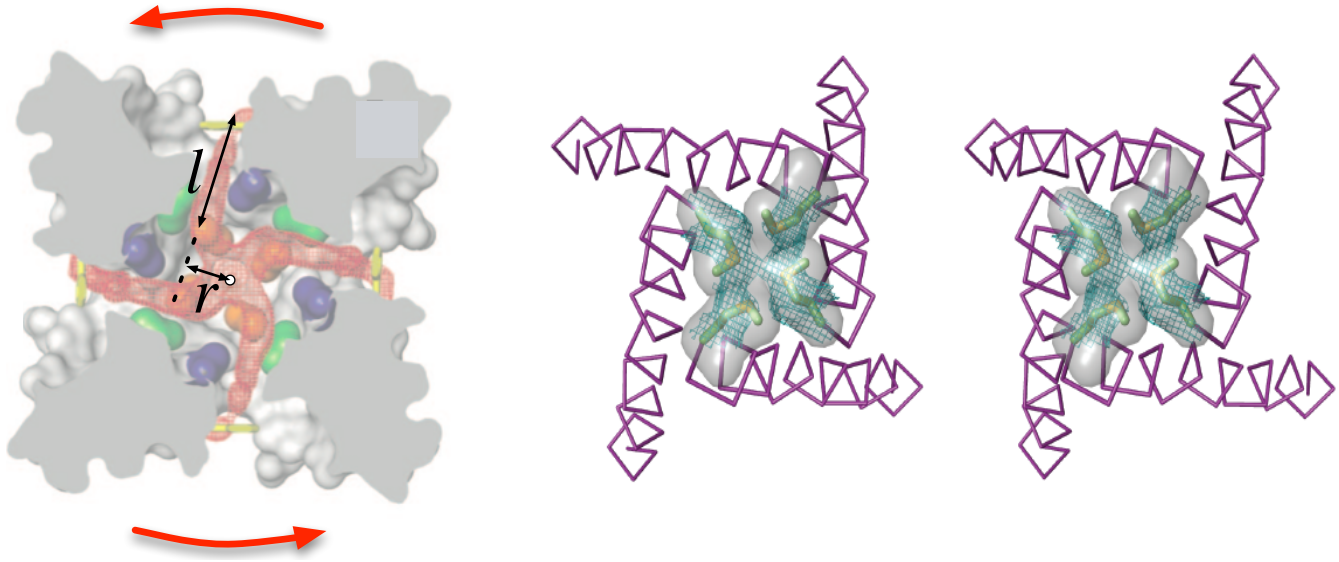

FIG. 3: Geometry of the rotor. (left) High speed jets of sodium ions and water exit the four portals, spinning the pore protein in the direction indicated. The angular momentum of the fluid flow depends on the length of the non-radial straight segment l, the moment arm r and the total mass flux through the pore. (right) Stereo viewgraph, adapted from the Supplementary Material of Payande et al [4]. (Presented for viewing with eyes crossed). View is same as left panel, from top of the protein. Note that counter-clockwise rotation of the helix corresponds to a downward flow of ions and water on the exterior of the protein.

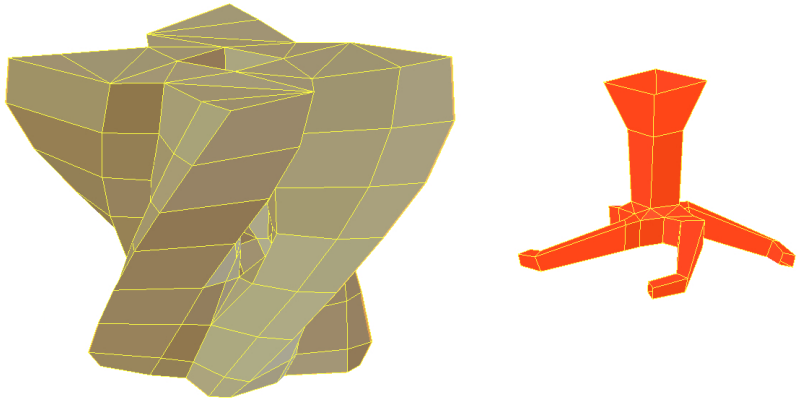

FIG. 4: Sketch of the exterior of the sodium channel protein (left), and the shape of the interior channel (right), through which a flow of sodium ions and water passes. Computer graphic courtesy of Chris Shaw. Sure looks like a rotor!

bottom of such a structure (Fig. 6), in another potassium channel protein. They observe variable twists of up to 23 degrees (perhaps 45/2?), and suggest that this motion might have a relation to channel gating. Their observation that the twist is variable may mean that, if channels spin, the rate of rotation itself is under biological control.

C. The "twisted barrel"

Among the most spectacular of the recent molecular images are those of Unwin and collaborators, of the "nicotinic acetylcholine receptor" of the electric organ of the marbled electric ray, *Torpedo Marmorata*. This organism feeds by shocking passing fish. Large voltages and currents are required to operate in the high conductivity salt water environment, these are generated in high-density arrays of channels called "electrocytes". The channels will naturally organize into a lattice, this tendency to crystallize allowed high-resolution Fourier analysis.

The resulting pictures showed what Unwin called a "twisted barrel" [7]. Later images indicate a more intricate structure, but to a first approximation the channel is constructed of five subunits, "in which each subunit

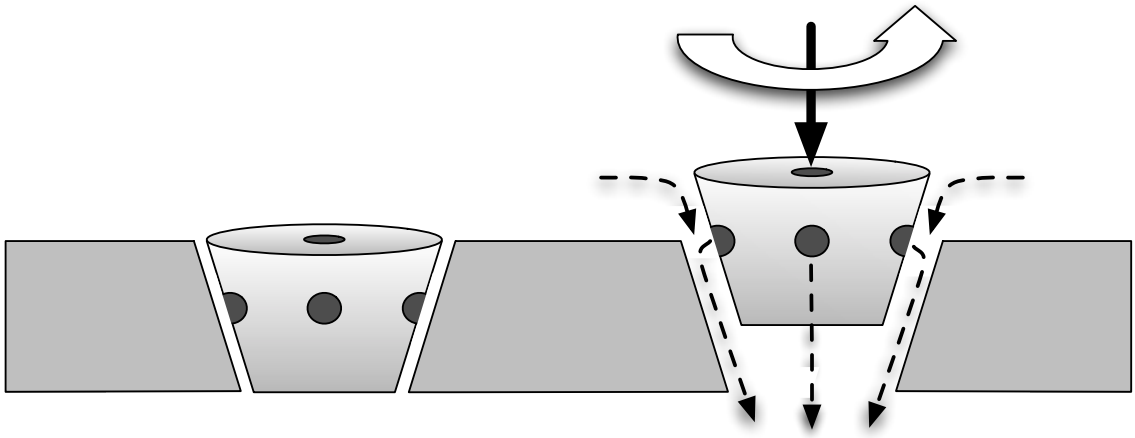

FIG. 5: Putative operation of a voltage-controlled sodium channel. The entire protein moves vertically, allowing spinning and lateral flow out the four windows, and down the sides to the cytoplasm. The diagram indicates the possibility that some flow bypasses the central pore altogether.

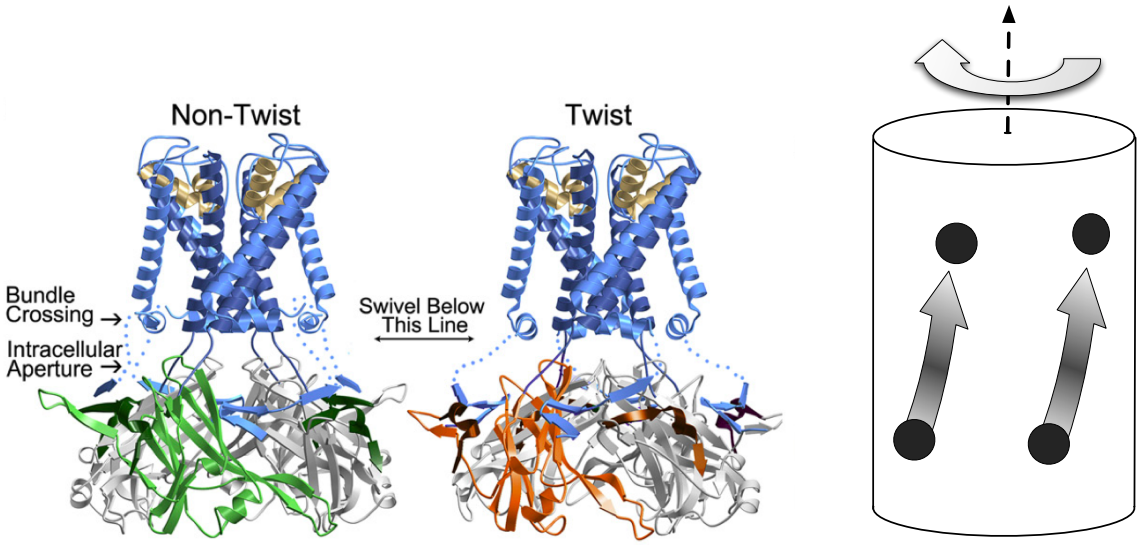

FIG. 6: (left) Diagram from Clarke et al [6]. A potassium channel, with similar architecture to Fig. 1. Twist angle between upper and lower portions of the protein is variable, up to 23 degrees. (right) Sketch indicating that a helical fluid flow in a cylinder (entering the bottom and exiting the top holes) will result in a rotation in the other direction of the cylinder itself. Rotation rate will depend on relative angle between upper and lower holes.

resembles the blade of a propeller" [9], see Fig. 7. Note that the angle of the blades is around 45 degrees.

This channel opens when the neurotransmitter acetylcholine binds to two specific sites on the protein. Sodium ions then pour through the channel, along a helical path, and as will be argued below, can exert considerable torque on the protein as a whole.

II. WHY THE ROTATION?

An open ion channel can easily pass 10^7 ions per second, this can be directly measured by patch clamp techniques. This is the same order of magnitude as movements expected in bulk diffusion; flow through an ion channel can be 30 times greater than through a simple pore like gramicidin A [1]. That such a flow though a single file pore could occur at these rates simply by unaided diffusion strains credulity, at least for some authors, see for example the discussion in Eisenberg [10].

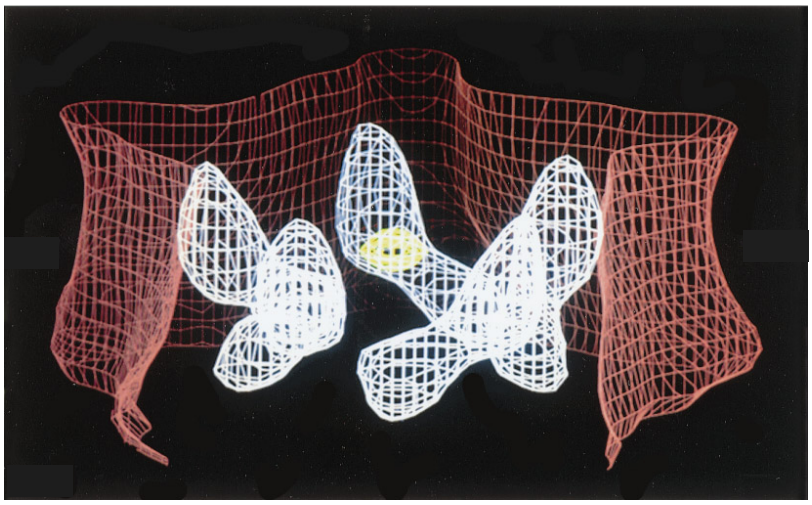

FIG. 7: Central pore of the open acetylcholine receptor channel, at 9Å resolution, from Unwin (1998) [7]. Sodium ions flow downwards, past five helical blades. Considerable torque on the unit as a whole can be generated.

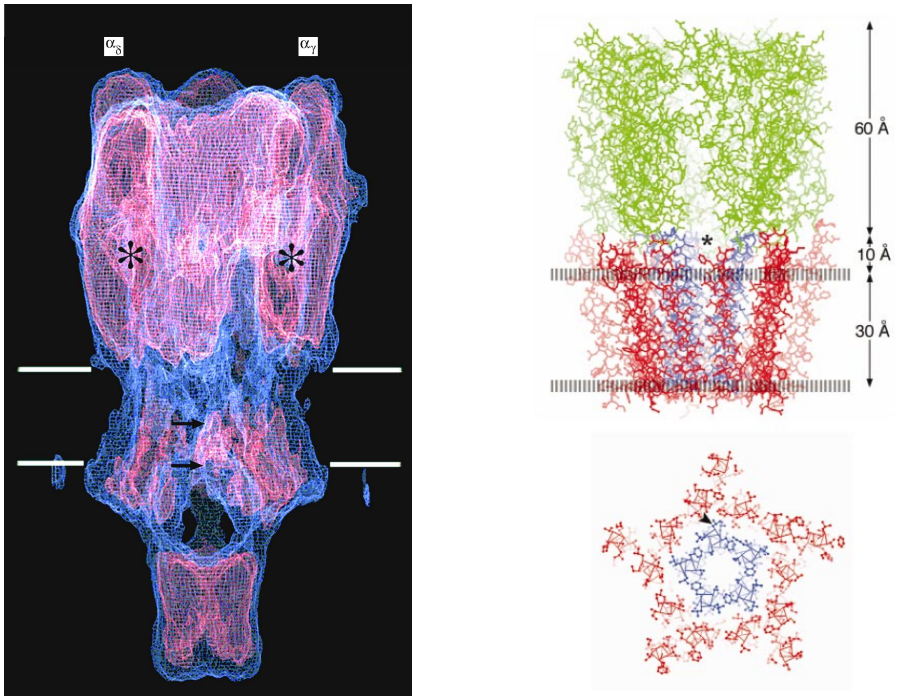

FIG. 8: Views of the acetylcholine receptor channel, at higher resolution. (left) Side view, asterisks indicate locations where acetylcholine molecules bind. *Both* sites must be occupied for the channel to be opened. From Unwin, (2000) [8]. (right) Side view, and a cross-section of the channel taken midway through the membrane. Water and ions are conjectured to enter at the asterisk (top), and to be present at the arrow (bottom). From Miyazawa, Fujiyoshi and Unwin (2003) [9].

If ion channels rotate, they presumably do so to increase ion flows across the membrane. But how can inserting a rotor in a simple open hole increase the net transport of ions? This will be a preliminary discussion, more careful work is in preparation.

Figure 9 presents a proposed explanation. On the left side of the figure, consider an ion resting on the vane of a rotor, free to turn. An electric field acts on the ion, producing a downward force. If the vane is at an angle, this force is resolved into a torque on the rotor as a whole, the torque is at a maximum when the vane is at an angle of 45 degrees. We can compute the torque, given the charge of the ion and the radius of the rotor.

However in a real ion channel, there are often many ions in the pore at one time, and few if any would be actually sitting directly on a vane. How can we estimate

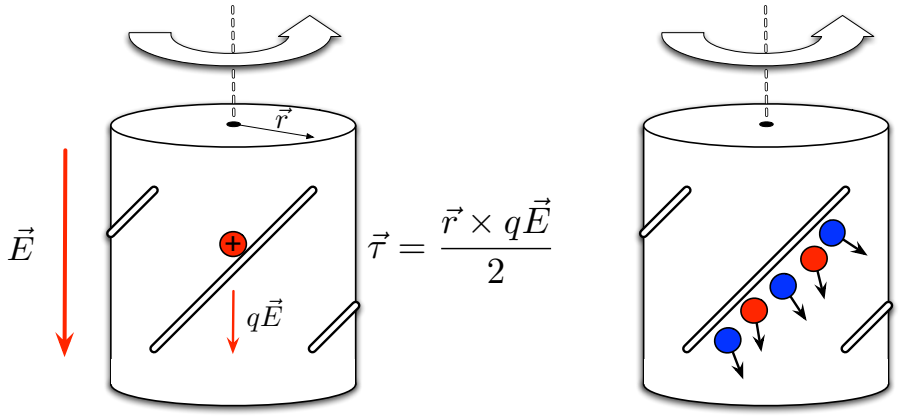

FIG. 9: A possible explanation why adding a rotor increases transport across a membrane. (left) An ion pulled by an electric field imparts a torque to the rotor. (right) The rotor efficiently propels ions and uncharged water molecules downwards. The net transport of the mixture is greater than that of a simple open pore of the same effective area.

the total torque on the rotor in this case?

The answer may be simple. The system is dominated by friction, an ion will experience many thousands if not millions of collisions as it passes through the pore. We expect first-order dynamics [10]. Consider weighing the system consisting of a marble sinking in a jar of honey. Once the marble has reached its terminal velocity, the system is in a "dynamic equilibrium", and its weight is the simple sum of the marble and the jar of honey, even though the marble is moving.

The rotor situation is similar, because of the large time-scale differences between dynamical relaxation processes in the fluid flow, and any likely rotor motions. A gas of hard disks, for example, relaxes to a local Maxwell-Boltzmann velocity distribution in only a few collision times, although diffusional spatial mixing takes much longer (unpublished computer simulations). The mean time between collisions in water is of the order of 10^{-13} seconds, thus we can expect relaxation to an equilibrium momentum distribution within a picosecond. Thus, from the point of view of the fluid moving through the pore, even a rotor turning at say 100 kHz acts as a nearly stationary boundary condition. So the stresses of any moving ions in the fluid will be collected by the rotor below, and converted to torques in a simple additive fashion.

Stated another way, momentum degrees of freedom relax essentially at the speed of sound, but spatial mixing is sterically hindered. Note that in this picture the torque on the rotor is independent of rotation rate. So the rotor would accelerate until its speed is checked by back pressure of the ion flow, or overall friction on the rotor.

The right side of the figure schematically indicates that the rotor can in turn impart momentum coherently to the fluid volume as a whole. This results in less dissipation within the fluid, and greater net transport. Momentum passes through the single degree of freedom of the rotor, and is added to the motion of in particular the uncharged water molecules, in the direction of the flow, in a much more efficient fashion than less oriented collisions with individual moving charged ions. In this sense, the rotor acts directly as a pump. Diffusion of isolated ions moving through water in a fixed channel would be much slower.

Admittedly, this is a central argument of this paper, and needs to be made more precise. A computer simulation is under construction.

Again, how can an added mechanical degree of freedom improve upon the diffusion occurring through a simple open hole? It perhaps seems like perpetual motion. But we must remember, this is not an equilibrium situation, and intuitions based on equilibria may not apply. For example, a rectifier, a highly nonlinear circuit element, can be constructed on the nanoscale which will operate at arbitrarily small potential differences [11–14]. Also, the work of Helbing et al [15] on the movement of crowds, as well as recent work of Zuriguel et al [16] on granular flow from a hopper demonstrates that placing obstacles in a diffusive flow can actually enhance the flow rate, in a strongly nonequilibrium situation.

It is worth noting that, even if the rotor were not free to turn, the torque imparted to the helical structure would be large, more than enough to produce conformational changes. To put in some numbers, and referring again to the left side of Fig. 9, consider the torque on a rotor with vanes inclined at the kinetic optimum of 45 degrees, produced by an ion of charge e, in an electric field E pointed downward. A voltage of 70 millivolts across a membrane of only 25 Å or so produces a very large electric field. Assuming a rotor radius of 10 Å, we obtain a torque of $\tau \approx 2.5 \times 10^{-14}$ dyne-cm per charge.

If the protein is not free to rotate, but is still subject to a rotational torque, bonds will presumably be deformed in response. We can estimate the amount of elastic energy stored as a result. Energy stored in a linear spring is $E = 1/2\ k\theta^2$, where k is the torsional spring constant. But we know that $\tau = k\theta$, and we have an estimate of τ from above. So $E = 1/2\ \tau\theta$, assuming a modest twist $\theta \approx 1$, we get $E \approx 10^{-14}$ erg, or of the order of kT stored energy due to the torque from just a single

charge. Many charges acting cooperatively would result in enough available energy to at a minimum produce conformational changes of the protein.

So the general picture is that part of the energy available in the form of the electric potential across the membrane is used to operate the rotor directly as a pump, increasing the flow of ions across the membrane. It might be objected that there is not enough energy available to overcome friction and spin a "heavy" protein, weighing about 300,000 daltons, at any significant speed. The question of "friction" at molecular length scales is problematical, but the available energy is enormous. A quick estimate shows that if even one millionth of the electric potential energy passing through the channel were diverted to pore rotational energy, the protein would accelerate to 1000 Hz in a microsecond. From this point of view, if there is any helicity at all in the protein structure, the question becomes not why the pore protein spins, but why it doesn't.

III. GATING AND ROTATION, THE "HANDBRAKE"

A central task of an ion channel is to gate ionic flows, to open, either in response to the presence of a particular molecule (a "ligand") such as acetylcholine (ACh), or a change in voltage across the membrane. Usually, pore openings are binary events, the pore is either completely open, or completely closed. Pores open and close stochastically, governed by a probability distribution which is influenced by, for example, the voltage across the membrane. The Hodgkin-Huxley equations, which give membrane currents as a continuous function of voltage, describe a macroscopic average over many pores.

If the argument of this paper is correct, the observed bistability of channel flow, whether a given channel is conducting or not, corresponds to a protein rotating or not rotating. Ligands or voltages would control "handbrakes" which halt the rotation by friction. The stochastic element of channel opening and closing could be described as a "stick-slip" regime of the rotor frictional environment. Again, the meaning of "friction" on these atomic scales is questionable, but perhaps some macroscopic intuitions are still useful.

A further possible advantage of the handbrake and rotor architecture is that more than one brake could act on the single central rotor. That way, different influences could act *in parallel* to slow or stop the rotor. For example, a brake more towards the intracellular part of the pore would correspond to an "activation gate", and another brake closer to the outside would correspond to an "inactivation gate". This positioning is in accord with the current understanding of ion channel physiology and structure [1].

A. ACh receptors, "handbrakes" on the rotor

The nictotinic ACh receptor requires the binding of *two* molecules of acetylcholine to open the channel. As sketched on the left in Fig. 10, in the nonconducting state two of the five subdomains are in a "distorted" conformation. But when acetylcholine binds to these sites, they relax to a conformation similar to the other three units. *Both* receptors must be filled for the channel to conduct. To quote Unwin and collaborators [20], "The conversion of the receptor to a more symmetrical state is therefore an additional, fundamental property of the activation mechanism". The interpretation in terms of the rotating channel picture is clear, acetylcholine retracts a pair of "brakes", allowing the rotor to turn, and the channel to conduct.

Unwin and collaborators describe changes in the pore configuration as the receptors are filled. But a recurring difficulty of trying to describe gating of this channel is the long distance (50 angstroms) from the receptor sites to the location where the actual gating is assumed to take place. As shown on the left in Fig. 8, the binding sites are up above the membrane at the asterisks, and the gates are presumed to be down at the membrane level, indicated by the two black arrows. So a long molecular linkage must be found; the handbrake model does not have this problem. Perhaps the two descriptions can be combined, when *both* brakes are released, the central portion can twist, and affect the pore geometry through a torsional conformational change.

Natural channel toxins, from spiders, snakes, poisonous plants, snails, frogs, and other creatures best avoided, have been invaluable in the study of ion channel function [1]. The action of several of these toxins can interpreted as freezing the "handbrakes" either on or off. Figure 11A and B, adapted from Hansen et al [18], show the effect of various toxins on a simpler ACh-type receptor, one with a rotor constructed of five identical units. "Antagonist" toxins jam the channel closed, with "C-loops" extended, as shown on the left of Fig. 11A, while "agonist" toxins pull the C-loops in, allowing the rotor to spin, and holding the channel in a conducting state.

The action of the Lynx spider toxin, illustrated in Fig. 11C is particularly easy to understand, if ion channels rotate. One end of the toxin attaches to the rotor, and the other end anchors to the membrane [19], thus directly preventing rotation. How this toxin might operate otherwise is obscure, as it attaches to locations far from both the ligand binding sites and the gate.

B. Voltage control and "handbrakes"

Exactly how voltage changes across the membrane are converted to ion flux changes, thus enabling traveling nerve impulses and all animal mischief, remains unclear. For one introduction, see Horn [21], and references therein and thereto.

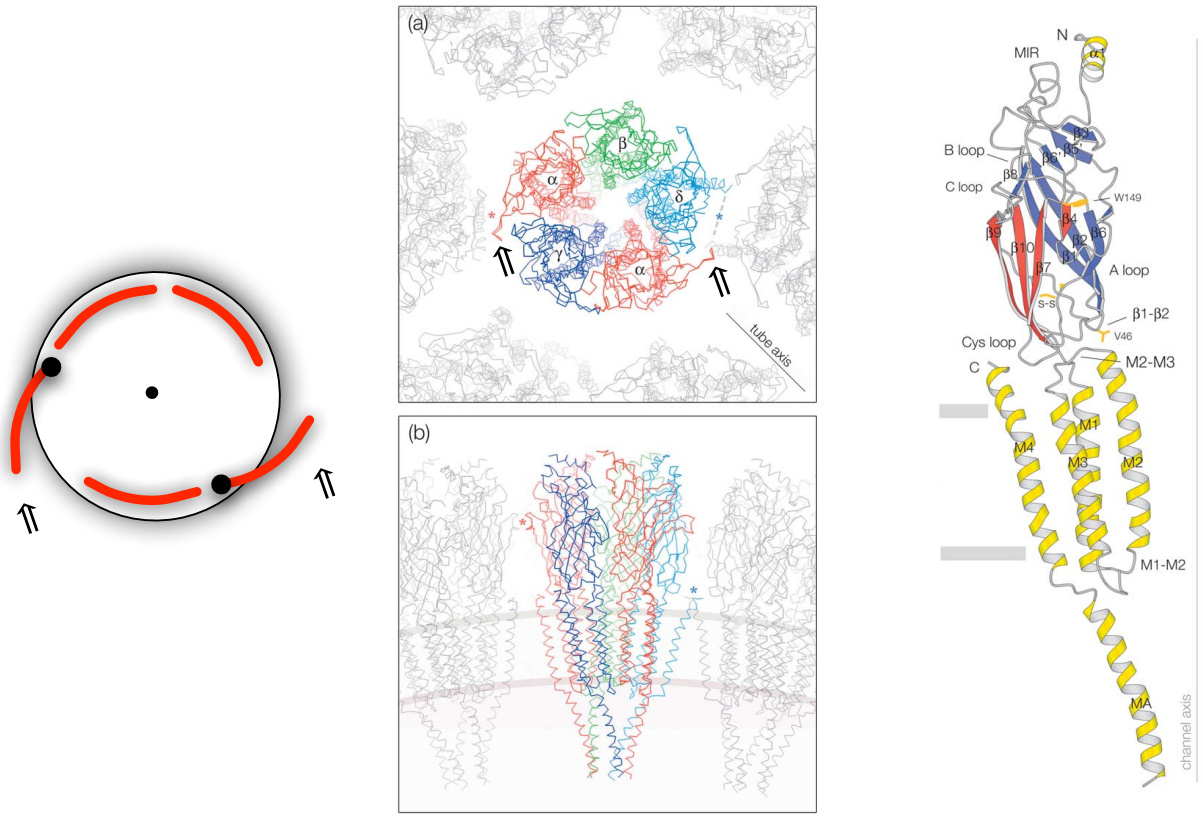

FIG. 10: (left) Diagram of "handbrakes", *both* must be retracted to enable rotation of the channel. (center) Arrows indicate location of "C-loops", which retract when acetylcholine binds to the receptor. (a) top view, (b) side view. (right) Structure of one of the pentamer units. Note that there seems to be no direct connection between the outer M4 - MA sheath and the rest of the protein. Center and right panels taken from Unwin (2005) [17].

Figure 12, adapted from Horn [21], sketches one prominent model. Charged "voltage control paddles" move in response to a membrane depolarization, and open the channel [22]. Many details of this model remain contentious issues. How exactly does the supposed "linker" at the bottom open the central pore [23]? Do the "paddles" move across most of the span of the membrane [22], or only a short distance [24]? Or do the paddles even detach completely when the channel opens [25]?

Though this is just one of several competing models, it is worth noting that the "paddles" are at the periphery of the pore protein, not next to the channel as might be expected for direct control of gating by a voltage sensor. Further, a proposed motion of the paddle which opens the pore is a retraction from an extended position to a more compact configuration. This is akin to the "handbrake" model discussed above, the channel can more easily rotate if extended arms are pulled in.

Figure 13 illustrates a top view of the "Shaker" voltage controlled potassium channel, according to Phillips and Swartz [26]. The four outer units, the putative charged voltage sensors, are attached by apparent hinges to the central putative pore. Phillips and Swartz argue, on the basis of mutation studies, that their data is best explained by a model in which the four "paddles" move independently, and that "the channel is open (conducting) only when all four voltage sensors move into the open position". In another mutation study, Gagnon and Bezanilla [27] disable two and even three of the "paddles" by neutralizing their charges, and find that the channel still functions, reacting to voltage changes across the membrane. Presumably the disabled paddles are in the "up" position ("up" and "down" are common terms in the literature for voltage sensors in respectively the open and closed position). Again, a model wherein all four handbrakes must be released to enable rotation and thus conduction is capable of explaining these results.

Much of the discussion of voltage gating appearing in the literature is perhaps somewhat suspect, for the following reasons. The standard picture [1], which goes back to Hodgkins and Huxley, is that the channel is opened in response to a voltage change across the membrane, by a conformational change. Some "gating charges" move in the electric field, "performing mechanical work" on the channel, and opening it. A thermodynamical treatment, assuming an equilibrium Boltzmann distribution and a fixed temperature, can relate the steepness of an observed voltage response curve to the number of gating

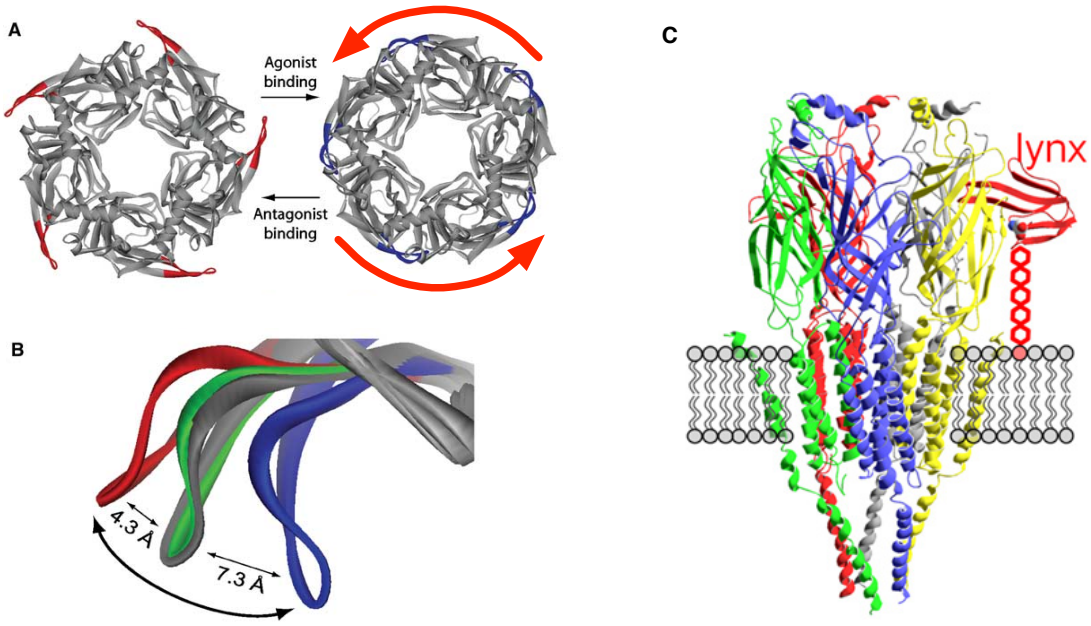

FIG. 11: Suggested mechanism of operation of various toxins on acetylcholine-type receptors. (A) top view, an "antagonist" toxin extends the C-loop "handbrakes", while an "agonist" toxin retracts them, allowing rotation in the direction indicated by the red arrows. (B) various positions of the "handbrake", fixed by toxins. red - α-conotoxin (sea snail) venom, an antagonist; blue - epibatidine (Ecuadorian frog venom), an agonist; green - lobeline ("indian tobacco"), a partial agonist. (C) side view, lynx spider venom. This toxin operates by attaching to the rotor, and dropping a "GPI" anchor to the lipid membrane, thus precluding rotation. Panels (A) and (B) adapted from Hansen et al [18], panel (C) taken from Miwa et al [19].

charges required. Numbers on the order of 10 to 15 elementary charges moving across the entire potential drop of the membrane are obtained, and this is often treated as a hard constraint in the literature [1].

But there is no reason to expect channel gating to be an equilibrium process. In fact, when the channel is open, a full picowatt can be dissipated in the near neighborhood of this supposed equilibrium. This is a large amount of power on a molecular scale, a picowatt can bring a 10 Å cube of water to a boil in under a microsecond.

By adding mechanical degrees of freedom to a system, one can manipulate flows of energy in ways not amenable to an equilibrium thermodynamics treatment. For example, when you stop a car going down a hill by applying the brakes, you are not actually "performing mechanical work" on the vehicle with your foot. The additional degrees of freedom allow one to control a large object with a small expenditure of energy.

IV. LOOKING FOR THE "SOCKET"

The membrane pore structures described above are proteins of around 300 - 400 kDa, with radii near 30 - 50 Å, floating in a lipid bilayer. As mentioned earlier, if such an object floats freely in a two-dimensional membrane liquid, classical arguments yield a rotational diffusion coefficient on the order of 10^4 second^{-1} [31]. This means that even without external driving, the object will be randomly rotating, due to thermal fluctuations. Average angular displacement will increase as the square root of time, and a meander of a full rotation is expected in a few milliseconds. An early experiment showed that the rhodopsin molecule turns freely in the frog retina [32], and the observed rotational diffusion was used to estimate the diameter of a rhodopsin [33].

However, sodium and potassium channels often do not move freely in the membrane, lateral diffusion does not take place. In neurons, they are tethered to the cytoskeleton in dense groups, at the axon initial segment, and in the nodes of Ranvier, and have been since around the time we became chordates, and, a short eon later, acquired a hinged jaw [34]. This localization would seem to immobilize the channels, and preclude the premise of this paper. But happily, a further exploration of the literature provides some intriguing detail, and a reprieve.

Connections between membrane proteins and the cell cytoskeleton are made via "ankyrin" proteins, first found in red blood cells [35]. Sodium channels in higher animals are localized by a particular type, "ankyrin-G". Now channels in general often do not occur in isolation, but rather as a part of a molecular assembly. For example it was found that sodium channels in mammals occur in association with "auxilliary β subunits" [36]. Subunit $\beta 1$ has been identified as the site to which ankyrin-G attaches at one end, and the spectrin of the cytoskeleton at the other, thus localizing the sodium channel. However the $\beta 1$ subunit is *not covalently bound* to the main α

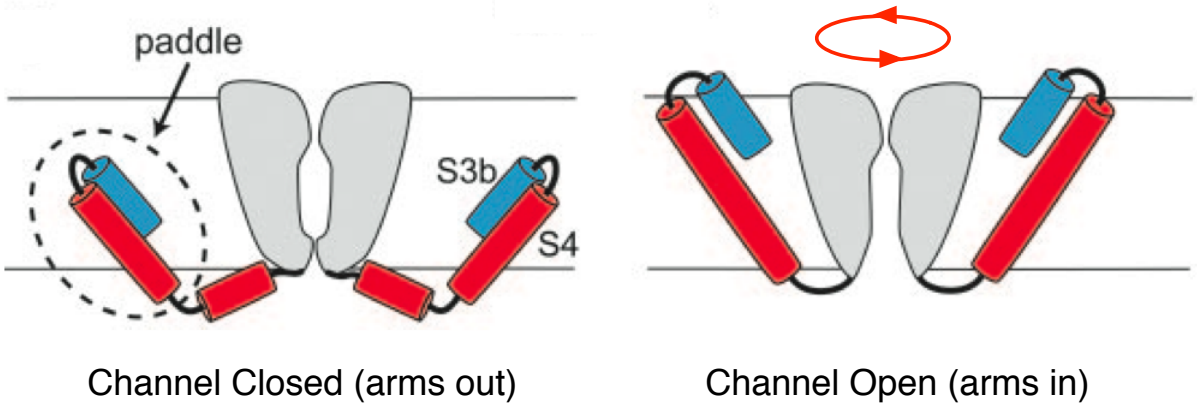

FIG. 12: Suggested operation of a voltage controlled gate. Changes in voltage across the membrane act on the charged paddles, and pull the arms upwards and inwards, releasing the "handbrakes", and allowing rotation. Figure adapted from Horn [21].

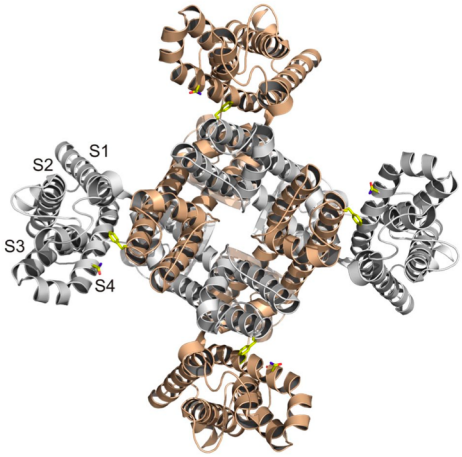

FIG. 13: Top view of the "Shaker" potassium voltage controlled gate, according to Phillips and Swartz [26]. The outer charged paddles move upwards (towards the viewer) when the channel is opened, and are loosely attached to the central pore. Choveau and colleagues suggest they might even detach completely [25].

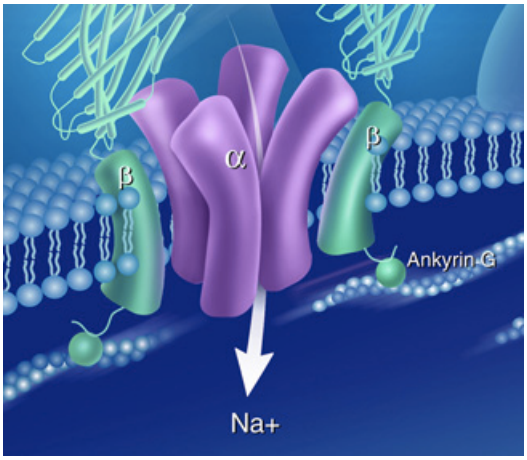

FIG. 14: Diagram of the possible sodium channel subunit architecture, taken from the Isom lab website [28]. There is no covalent bond between the β and α subunits, the latter is free to turn. The helical nature of the α subunit is not represented here.

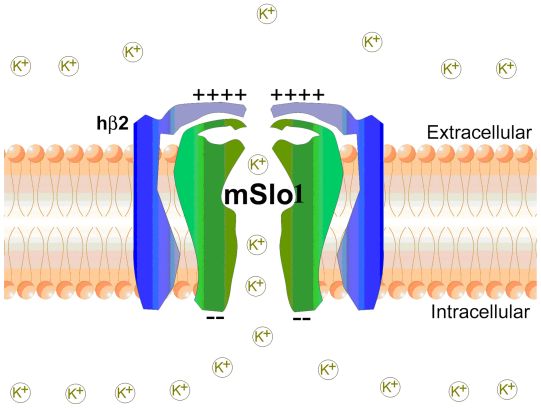

FIG. 15: Diagram of a possible potassium channel subunit architecture, taken from Chen et al [29]. Once again, a β subunit might serve as a socket allowing the interior rotor to freely turn.

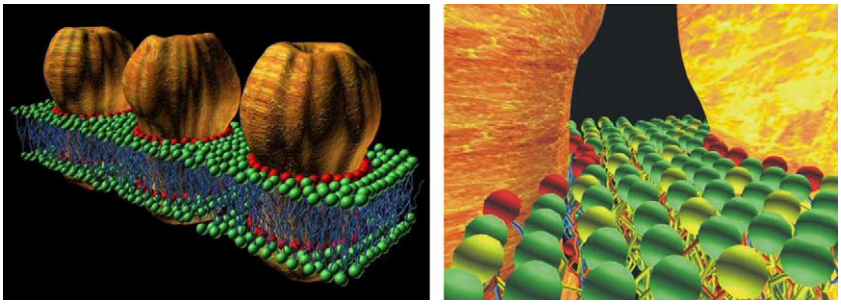

FIG. 16: Impression of an array of acetylcholine receptors, taken from Barrantes [30]. Proper functioning of these channels requires at least one layer of surrounding lipids, indicated in red. The lipids could "lubricate" rotation or twisting.

subunit. Figure 14, taken from the Isom lab website [28], illustrates the situation. From the point of view of this paper, subunit $\beta 1$ is a *socket* within which the α unit spins.

The study of "subunits" for potassium channels is also rapidly advancing [37]. Particularly evocative in the context of a possible "socket" is Fig. 15, a diagram taken from work by Chen et al [29].

Likewise the nicotinic acetylcholine channel (NACh) does not float freely in the membrane. In the electric ray, the channels occur in a lattice, and are localized by their neighbors. In muscle, the channels are localized in the neuromuscular junction. In either case, lateral motion is impossible, so we must look for some sort of sheath within which the protein rotates.

Unwin and coworkers crystalized a lattice of NACh units, presumably similar to that of the in vivo electrocytes of the electric ray. This lattice is thought to be fixed by disulphide bonds between neighboring units [38] (the blue asterisk in the lower center of Fig. 10). Thus for rotation within this constraint, we either have to explain away the disulphide bridge in Fig. 10, or look for a socket. There does in fact appear to be a candidate. A study of Unwin's work suggests a division between parts of the protein that rotate, and parts that are stationary.

The right panel of Fig. 10, from Unwin, shows one of the five subunits, apparently divided between an interior part, featuring chains M1 through M3, and an exterior part, chains M4 and MA, in Unwin's notation. Now it has been generally assumed that all the chains, and all the subunits are conjoined into a single unit. But a detailed reading of Unwin's admirably careful work suggests the possibility that in fact the interior part might be able to move relative to the exterior part, which is in contact with the lipid membrane. If one looks for direct evidence for a solid connection between the M4-MA portion (putative socket) and the M1-M3 portion (putative rotor), one find comments like [9, 17] "Helix M4 is less precisely positioned ... and comes away from the others, by variable amounts, at its extracellular end." "Part of the M3-M4 loop (connecting MA to M3) is missing." "Most of the rest of M3-M4 (i.e. M3-MA) appears to be disordered and is not seen in the structure." "However, the description of these regions may be incomplete, given that parts of the M3-MA loop may be involved that are not visible in the structure."

In short, there is enough uncertainty in the connection to allow, at a minimum, continued wishful thinking.

An alternative possibility is suggested by work reviewed in Barrantes [30]. In this picture, schematically illustrated in Fig. 16, NACh receptors are still closely packed, but float either in a natural or a reconstituted

lipid membrane. The emphasis of the Barrantes article is that the surrounding lipids are absolutely necessary for the proper function of the acetylcholine receptor. In fact, at least one full layer of lipid (about 45 lipid molecules) must surround the receptor protein for it to function [39].

Additional support for this picture is supplied by a number of recent papers, e.g. [40], which note the "surprising" fact that mutations affecting the structure of M4, out towards the lipid membrane boundary, can have a large effect on the behavior of the pore. Typically, changes at the outer edge of the protein affect the statistics of opening and closing, but not the actual magnitude of the flow through the pore [41]. These experimental facts are not surprising, and are in fact to be expected, if NACh receptors are rotating.

V. ROTATION RATE ESTIMATES, MECHANISMS

An interesting feature of the (presumptive) architecture of the Payande et al sodium channel model, as well as other models, is that the fluid paths are separated into lateral portions, perpendicular to the electric field, and thus not accelerated by it, and vertical portions moving through the vanes of a rotor. We have possibly two functionally separate structures, mounted on a single shaft, like the compression and exhaust turbines of a jet engine.

In the case of motion perpendicular to the field, we can consider conservation of angular momentum of this part of the flow, in isolation. This gives a rotation estimate which is just a function of the flow velocity, independent of any applied field. In the second case, the electric field does act on the fluid, and imparts a torque to the rotor. This torque will accelerate the rotor, until opposing forces rise enough to maintain a steady terminal velocity.

Again, the system is dominated by friction, we expect first-order dynamics [10]. Estimates of the directed rotation rate of a pore will be superposed on the random thermal rotary motion. Numbers are taken from Hille's inescapable reference [1].

To begin, let's consider the angular momentum of the horizontal plate with spiral channels of Fig. 3, perpendicular to the field, in isolation. Despite the thermal buffeting, and high dissipation, overall angular momentum will be conserved. Even toothpaste squeezed through this spiral path will produce a counter rotation, if the plate is free to turn. So all we need do, if the protein is free to turn, is compute the change in angular momentum of the fluid flow around the protein axis, and apply it in the opposite direction to the protein itself. If the flow were strictly radial, there would be no angular momentum associated with it. But as diagrammed in Fig. 3, the fluid flow takes a bend a short distance from the center, and there is a net moment around the axis, if we take this picture of the flow pattern seriously.

Angular momentum referred to some axis is $r \times p$, where p is the linear momentum, and r is the vector from the center of rotation. The relevant linear momentum is contained in the fluid not moving radially, the portion of length l in the figure. This momentum is estimated from the ionic flow rate. Assume an open sodium channel current of 10^7 ions per second. To this flow we must add the flow of associated water molecules. This number is much less clear, for some potassium channels it is thought to be only one or two water molecules for each K^+ ion [42, 43]. But for higher volume sodium channels one might infer that there must be at least several water molecules per ion passing though the pore. Otherwise, reassembly of the hydration shell at the exit of the pore would require a considerable counterflow of water, which would be inefficient. So just to pick a modest number, let's say the flow through the pore has a mass flux $F \approx 10^9$ Daltons/second, corresponding to four waters for each Na^+ ion.

The angular momentum of this flow does not depend on the cross-sectional area of the tubes, or the fluid velocity, or the number of outward channels, these factors cancel out. We need know only the length of the non-radial part of the channel, and the moment arm, we obtain $L = F * l * r$. Taking a moment arm of 2Å and a non-radial channel length of 5Å (see Fig. 3) we estimate $L = 10^7$ kDa Å^2/sec.

Now the required counter-rotation of the protein pore is given by $L = I\omega$, where I is the moment of inertia of the protein. Let's assume a cylinder of mass 400 kDa, and radius 7Å. We have $I = mr^2/2$, plugging in the numbers, and using the value for L we obtained above, we get $\omega = 1000$ radians/sec, or a rotation frequency on the order of 150 Hz.

We have been assuming that the fluid velocity is much greater than any movement of the channel walls. Assuming that ions move single file across a 25Å membrane at a rate of 10^7 per second, we get a velocity of 2.5 cm per second! The model architecture of Fig. 5 suggests that there may be ways to defeat the single-file requirement, but even dividing by one hundred, we have a furious jet on the nanoscale.

The preceding spinning rate was estimated considering only the momentum, the mass transport of the stream of ions. But the energy involved in this process is rather small compared to the energy available to ions moving across the electric field on the membrane. We've argued that a large torque can be generated by a helical structure, and that this torque is to a first approximation independent of the rotor rotation rate.

So what does oppose the electrical torque, so that the rotor does not accelerate indefinitely? First, note that the reaction torque due to accelerating the mass of the ion-water mixture downward is negligible, the electrical forces completely dominate any forces required to change the momenta of the fluid stream.

The rate-limiting step possibly is the pressure gradient set up across the pore. If we have a spinning rotor, ions and water must be forced into a local pressure max-

imum, and near the entrance to the pore, a local relative vacuum is created. This suction could act to accelerate ions towards the entrance of the channel (suggestion of S. Still). At some rotation rate, the work required to move fluid across this gradient may become comparable to that available from the torque due to the electric field. The writer is fairly clueless as how to estimate these values, a computer simulation may provide more insight.

Another effect which would limit rotation is simple frictional drag, as the protein turns in the membrane. The classical discussion of Saffman and Delbrück [31] allows an estimate of the frictional torque of a cylinder floating in a lipid membrane. We expect the angular velocity to be related to an imposed torque by a "mobility", $\omega = b\,\tau$, in the linear regime near equilibrium.

Via Stokes we have $b = 1/(4\pi\mu r^2 h)$ where μ is the viscosity and r and h are the radius and height of the cylinder. Via Einstein we have $D_r = b\,kT$ where D_r is the rotational diffusion coefficient, thought to be around 10^4 sec^{-1} for a large protein floating in a membrane. So with $\omega = \tau D_r/kT$, and using the torque generated by a single charge, plugging in the numbers, we have, at room temperature, $\omega \approx 10^5$ rad/sec, or around 15,000 Hz.

There typically will be multiple charges in a pore, which would produce even higher rotation rate estimates. However, membrane protein shapes are clearly not purely cylindrical (Figs. 1,2,6,8), thus the frictional response may well be larger, and more complicated. There is no reason to expect why extrapolating the friction from the linear Onsager regime to spin rates of 15 kHz and more would be valid.

A final rough estimate is obtained by just assuming that we indeed have a rotor, and that it turns approximately with the mean speed of the flow past it. If we have a flow of 10^7 ions per second, each would pass through the membrane in 100 nanoseconds. If the path of an ion were to remain strictly vertical, and undeflected by the rotor, the rotor would have to turn at a certain rate to "keep up" with the flow. Again referring to the geometry of Figs 2, 3 and 4, the rotor would have to turn 90 degrees in that time. That gives an extraordinary rotation rate of around 2.5 MHz. Presumably the fluid path *is* deflected by the rotor, so this would be an upper bound.

Of course, these are crude estimates. But they do at least show that rotations driven by the electrochemical potential across the membrane are physically possible. If rotations actually occur, presumably the channel geometry and flow patterns are optimized to produce the best rotation, under constraints of total channel length, moment arm, and any number of other factors. The nanoscale images give us what the answer is, we just have to figure out the questions!

VI. ADVANTAGES OF ROTATION

Given the notion of a rotating channel, one can imagine many advantages of such motion for a living cell. At the risk of presenting only an "alternative reality biophysics", here is a summary list of speculations.

a. Increased net transport As argued above, inserting a rotor in a simple open hole might in fact increase the net flow of ions across the membrane. This, if it exists, is a strong nonequilibrium effect.

b. Part of a gating strategy Also as argued above, viewing gating as a mechanism of brakes acting on a rotor has advantages of simplicity, and not requiring any particular minimum gating charge for operation. The usual equilibrium treatment of gating theory may not be particularly relevant.

c. Rotor as agitator One function of spinning "baskets" and "gondolas" may be as simple as promoting diffusion by distributing (or picking up) ions over a wider area than what is possible with a simple passive pore. The cytoplasm is a complicated material, a local measurement of rotational diffusion of some molecules shows a viscosity not much larger than water, but lateral translation of large molecules can be hindered by factors of thousands [44]. Direct mechanical, or electromechanical agitation in the style of a washing machine might serve to overcome local barriers to diffusion, which may exist in particular near the cell membrane boundary [45].

It might be objected that fluid flow on small length scales corresponds to low Reynolds number, where "stirring" does not imply efficient "mixing" [46]. But on scales so small that the spatial extent of molecules comes into play, quite different physics arises.

d. Communication between pores Naundorf et al convincingly argue [47] (at least I'm convinced) that observed action potentials are better explained by a model in which ion channels communicate with each other, rather than the Hodgkin-Huxley model, in which pores are independent, and are affected only by an overall membrane potential. Further, within the Hodgkin-Huxley model, 85% of the electrical energy used in depolarizing and repolarizing the membrane is wasted [48]. Just from an energetics point of view, it would be much more efficient if, say, the opening of a potassium channel was coordinated with the closing of *local* sodium channels. The image of spinning rotors emphasizes the point that the membrane is a highly integrated and optimized system, and perhaps makes more plausible the idea of communication between pores.

e. Rectification The cylinder of Fig. 9 is symmetric in the vertical direction, and would conduct equally well in the opposite direction. But the structure of biological ion channels breaks this symmetry, and many channels can act as rectifiers, allowing flow in only one direction [1]. There have been a number of suggested rectification mechanisms, the pinwheel of Payende et al [4] (Figs. 3 and 4) adds one more.

Consider Fig. 16, taken from Ernst Mach's 1883 "The Science of Mechanics" [49]. This shows a pinwheel rather

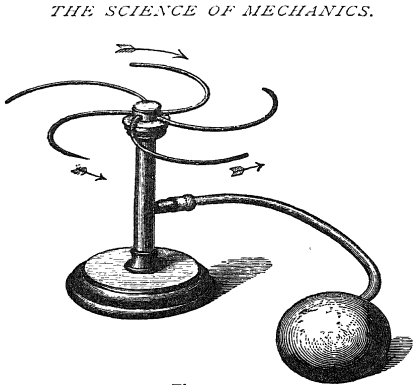

FIG. 17: Mach's 1883 "reaction wheel" acts as a rectifier. Air blown out the spokes of the wheel will cause a clockwise rotation, but the same amount of air sucked into the spokes produces no such rotation, in either direction.

similar in geometry to the Payende description. If we imagine Mach's "reaction wheel" operating in air, or a lawn sprinkler operating under water, the experimental observation is, forcing fluid out the jets causes the rotor as a whole to turn, while sucking fluid back in does not in general produce continuous rotary motion. Now physicists have been arguing about this problem since at least the time of Mach, see the article by Jenkins for history, and a discussion of the effect [50]. A quick explanation is to note that the inward flow pattern is not a simple reversal of the outward; in the inward case, the net pressure gradients on the rotor cancel, there is no net torque on the rotor. The end result of this geometry is perhaps to produce a rectifier, the rotor turns if fluid flows downward into the cytoplasm (in the case of sodium channels), but does not turn if there is a gradient in the other direction.

Installing a "ratchet" in the channel might speed ionic flow. So the picture is (wave hands vigorously), when there is a field across the membrane, the sodium ions are directly driven downward. But even when the membrane is depolarized, the rotor could continue to turn, as the spiral channel structure rectifies fluctuations in concentration. Though this view clearly needs refinement, there is no problem with the Second Law, as the system is not in equilibrium. The energetics, the direction of flow, is given by the Nernst-Planck equation, but the *rate* of flow is the result of a nonequilibrium process.

The study of the dynamics of electrolyte solutions is a difficult field [51, 52], not to mention the many unknowns of the material properties of cell membranes. Considerable modeling effort may be required to promote any of these speculations to "theory" status.

VII. CAN THESE ROTARY MOTIONS BE OBSERVED?

This paper perhaps has assembled a quantity of strong but entirely circumstantial evidence for pore rotation, there is no definitive proof. Also, much of the phenomenology could be explained in a picture not of full rotations, but partial twisting, under the influence of torques caused by fluid flow.

There is no substitute for experimental confirmation, especially when the rate of rotation, if present, is so unclear. The rotary motion of ATP synthase was directly visualized by attaching a piece of fluorescent actin to part of the rotor [53]. Perhaps something along these lines could be done for pore proteins. Another possibility is suggested by the work of Mannuzzu et al [54] and Cha and Bezanilla [55]. They were looking for movements of the "gating charge" by fluorescently tagging certain residues of the pore protein, and then looking for fluorescent signals in a membrane containing many pores, functioning under physiological conditions. If the pores are actually rotating, some average rotation of polarized light might be observed, since all pores are presumably turning in the same direction.

I will happily present a bottle of fine scotch to the first people to do this.

VIII. "STIRRING" CONCLUSION

We have argued that the nanoscale pictures alone, particularly of the sodium channel, make a rather compelling case for rotary motion of the entire pore. At a minimum, flow through a helical geometry can generate enough torque to provoke substantial conformational changes. The shaped proteins act more like a nanofluidic device than a mechanical gate. Besides the notion of rotary motion, speculations presented here include the suggestion that significant ion flow may occur outside the pore protein itself. If any of this is the case, it raises more questions than answers, and requires a complete rethinking of the dynamics of membrane pores.

People say that the easiest person to fool is yourself [56]. But if all this is a delusion, at least it's a remarkably detailed and entertaining one. Though maybe it's true! Some turn left, some turn to the right, they whirl together, floating on a tremulous sea of noise.

Acknowledgements

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