

Supplementary Material

Here we supply some additional material justifying the model construction and details on how the numerical computations were carried out.

Topology of the α Subunit and the Rotor-Stator Assembly

Fig. 9 shows the topology and amino acid sequence of the α -subunit from *P. modestum*. Considering the α -helical packing, it is likely that the rotor-stator interface consists of at least three stator helices; the pattern of charged and polar residues is consistent with the putative structure shown in Fig. 10. The rotor-stator interface must present a hydrophobic barrier against leakage of ions from the periplasm to the cytoplasm; thus the ion channel should not penetrate all the way through the stator, so that there is no direct path connecting the periplasm with the cytoplasm. To accommodate these constraints we propose the rotor-stator model shown in Fig. 11.

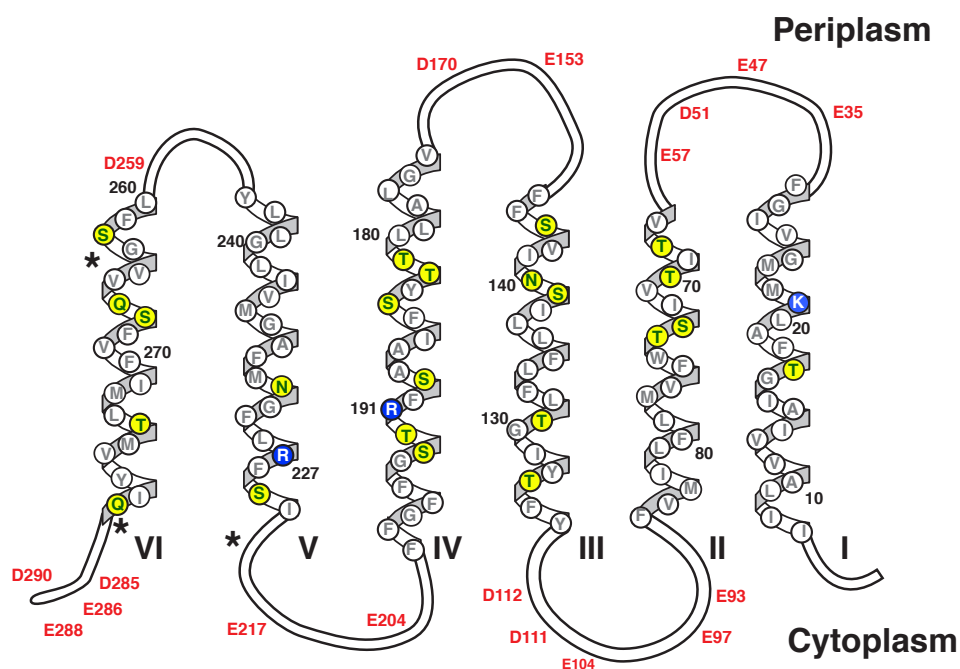


Fig. 9. Amino acid sequence of the α -subunit showing the acidic, basic, and polar residues. The essential stator charge (R227) is on helix V.

THE SODIUM F-ATPASE: SUPPLEMENTARY MATERIAL

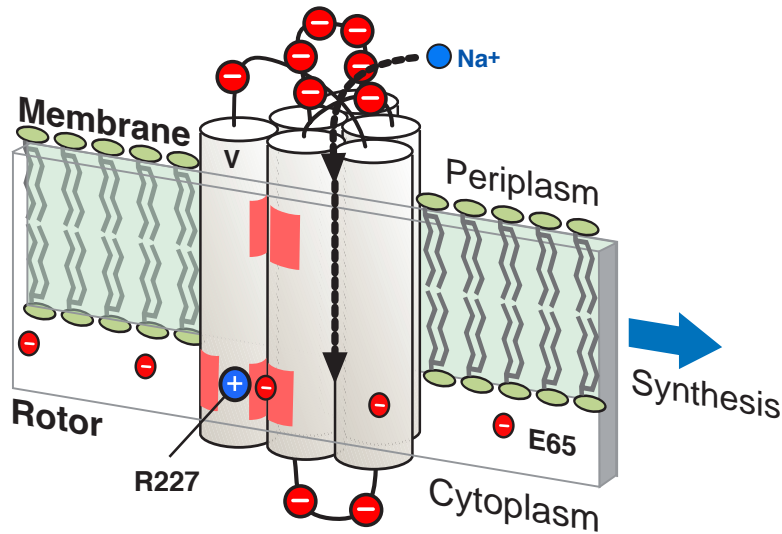


Fig. 10. Detail of the rotor-stator assembly model as viewed from inside the rotor, showing the location of the essential rotor and stator charges and the path of the sodium ion into the stator helix bundle. The ion channel terminates before penetrating all the way through the stator. Red patches indicate polar hydrophilic regions.

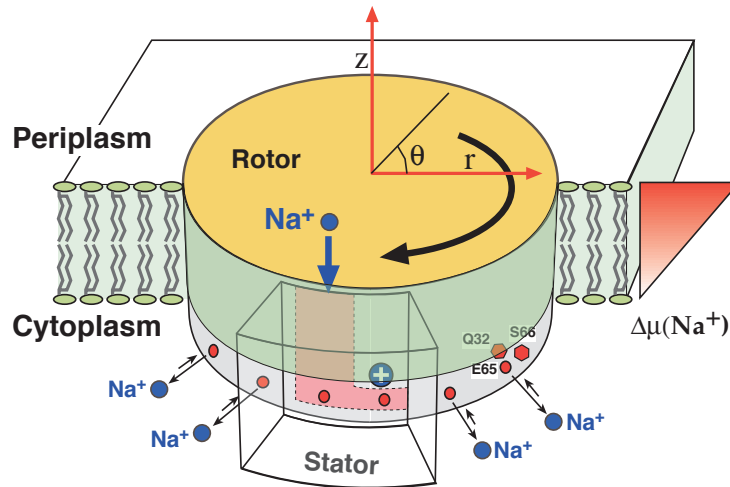


Fig. 11. Schematic of the rotor-stator assembly in *P. modestum* as viewed from outside the rotor. To formulate the mathematical model, a cylindrical coordinate system is established as shown. During ATP synthesis, the rotor turns to the left (clockwise viewed from the periplasm). The rotor section below the level of the membrane contains the 12 ion-binding sites. Each site consists of the triad Q32/E65/S66, which coordinates a sodium ion. Mutation of the ion-binding triad switched the coupling ion from sodium to lithium to protons (1). This strongly suggests that the association between the ions and the rotor sites is strictly electrostatic and can be modeled as a simple Coulomb well as we have done here.

Markov Chain Model for the Evolution of the Rotor State

The ionization state of the rotor-stator assembly is specified by the ionization states of the rotor sites, each of which may be ionized or empty. Because the rotor has 12 ionizable sites, the general chemical state of the rotor can take $2^{12} = 4096$ different values. However, rotor sites far away from the rotor-stator interface do not affect the rotor-stator interaction. Only the 4 rotor sites near the rotor-stator interface interact with the stator, and so specifying their state is sufficient to describe the rotor-stator interaction. Therefore, we define the chemical state of the rotor-stator assembly, denoted by s , as the ionization state of the four rotor sites near the rotor-stator interface. Thus there are $2^4 = 16$ possible chemical states.

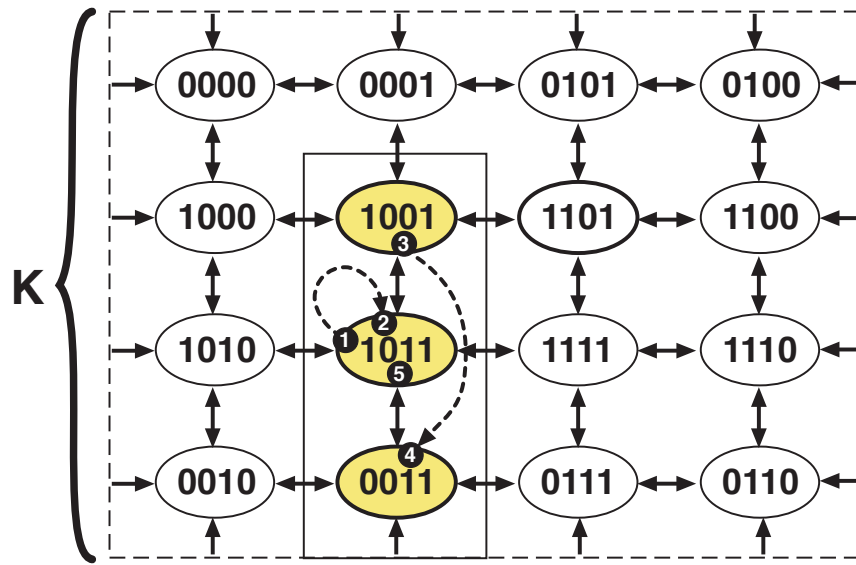


Fig. 12. The Markov transition between the $2^4 = 16$ rotor chemical states can be drawn on a two-dimensional torus. The 16 chemical states are numbered using a binary system as (0000), (0001), ... and (1111), where we denote an empty (charged) site by 1 and an occupied (neutral) site by 0. The top of the dashed rectangle is identified with the bottom and the left side with the right. Thus a transition exiting from, say, state (0100) to the right enters state (0000) from the left; if the same state exits upwards, it enters state (0110) from the bottom. Rotations are indicated by dashed lines. The numbers correspond to the sequence of events shown in Fig. 3.

The 16 states can be organized on the surface of a torus; this is shown schematically in Fig. 12. For the convenience of mathematical discussion below, we rename the 16 chemical states as state 1 through state 16. The sequence number, N , of the state $(i_1 i_2 i_3 i_4)$ is given by

$$N = i_1 \times 8 + i_2 \times 4 + i_3 \times 2 + i_4 + 1 \quad [1]$$

THE SODIUM F-ATPASE: SUPPLEMENTARY MATERIAL

Transitions between states occur when a site binds or releases a sodium ion. Since the relaxation to equilibrium after a proton association/dissociation event is much faster than the mechanical motion of the rotor, we can treat the transitions between these states as a Markov chain (2). Because of the electrostatic interactions between the rotor sites and the stator charge, the transitions between states depend on the angular position of the rotor, denoted θ .

Let $\rho(t, \theta; k)$ be the probability at time t that the system is in the k th state while the angular position of the rotor is fixed at θ . Note that $\rho(t, \theta; k)$ is *not* the probability density at time t that the rotor is at the angular position θ and the system is in the k th state; here θ is a parameter. By fixing the angular position of the rotor, we are able to isolate the kinetics and study its governing equation. In the subsequent sections, we will combine the kinetics and the dynamics to build a complete model for the sodium F_0 motor.

The governing equation for the evolution of $\rho(t, \theta; k)$ is

$$\frac{d}{dt} \begin{bmatrix} \rho(t, \theta; 1) \\ \rho(t, \theta; 2) \\ \vdots \\ \rho(t, \theta; 15) \\ \rho(t, \theta; 16) \end{bmatrix} = \mathbf{K}(\theta) \cdot \begin{bmatrix} \rho(t, \theta; 1) \\ \rho(t, \theta; 2) \\ \vdots \\ \rho(t, \theta; 15) \\ \rho(t, \theta; 16) \end{bmatrix} \left. \vphantom{\begin{bmatrix} \rho(t, \theta; 1) \\ \rho(t, \theta; 2) \\ \vdots \\ \rho(t, \theta; 15) \\ \rho(t, \theta; 16) \end{bmatrix}} \right\} 16 \text{ components} \quad [2]$$

where the transition matrix $\mathbf{K}(\theta)$ is a 16×16 matrix given by

$$\mathbf{K}(\theta) = \begin{bmatrix} -\Sigma_1 & k_{2,1}(\theta) & \cdots & k_{15,1}(\theta) & k_{16,1}(\theta) \\ k_{1,2}(\theta) & -\Sigma_2 & \cdots & k_{15,2}(\theta) & k_{16,2}(\theta) \\ \vdots & \vdots & \ddots & \vdots & \vdots \\ k_{1,15}(\theta) & k_{2,15}(\theta) & \cdots & -\Sigma_{15} & k_{16,15}(\theta) \\ k_{1,16}(\theta) & k_{2,16}(\theta) & \cdots & k_{15,16}(\theta) & -\Sigma_{16} \end{bmatrix} \quad [3]$$

In the transition matrix $\mathbf{K}(\theta)$, $k_{i,j}(\theta)$ is the transition rate from the i th state to the j th state; the diagonal entry Σ_i is given by

$$\Sigma_i = \sum_j k_{i,j}(\theta) \quad [4]$$

The transition rate $k_{i,j}(\theta)$ is nonzero only when the i th state and the j th state can be connected by the single event of a sodium ion binding onto or dissociating from a rotor site. For example, the first row of the transition matrix $\mathbf{K}(\theta)$ has four nonzero off-diagonal elements:

THE SODIUM F-ATPASE: SUPPLEMENTARY MATERIAL

- $k_{2,1}(\theta)$: transition rate from state 2 = (0 0 0 1) to state 1 = (0 0 0 0).
- $k_{3,1}(\theta)$: transition rate from state 3 = (0 0 1 0) to state 1 = (0 0 0 0).
- $k_{5,1}(\theta)$: transition rate from state 5 = (0 1 0 0) to state 1 = (0 0 0 0).
- $k_{9,1}(\theta)$: transition rate from state 9 = (1 0 0 0) to state 1 = (0 0 0 0).

The Interaction Between the Rotor and the Stator

To describe the geometry of the rotor-stator assembly, and to calculate the electrostatic interaction between the rotor and stator, we first establish a cylindrical coordinate system as shown in Fig. 11. The z-axis goes through the center of the rotor and is perpendicular to the plane formed by 12 binding sites. The circle of rotor binding sites is defined as $z = 0$. (r, θ) measures the radial distance from the z-axis and the angular distance to a reference line. Fig. 13 shows the rotor stator assembly projected onto θ -z plane and viewed from outside the rotor. The radius of the rotor is taken as 3 nm. Thus the circle of the rotor binding sites has coordinates:

$$r_{\text{site}} = 3.0 \text{ nm} ; \quad z_{\text{site}} = 0.0 \text{ nm} \quad [5]$$

We use the angular distance between two adjacent rotor binding sites as a unit displacement, denoted by $\Delta\theta = 2\pi/12$. The four parameters describing the geometry of the rotor-stator assembly in Fig. 13 are

$$\begin{aligned} \theta_1 &= -1.14 \times \Delta\theta \\ \theta_2 &= -0.84 \times \Delta\theta \\ \theta_3 &= 0.5 \times \Delta\theta \\ \theta_4 &= 1.0 \times \Delta\theta \end{aligned} \quad [6]$$

The positive stator charge, R277, is offset from the circle of rotor binding sites by 0.6 nm in the r direction. It has the coordinates

$$\theta_{\text{R277}} = 0.75 \times \Delta\theta ; \quad r_{\text{R277}} = 3.6 \text{ nm} ; \quad z_{\text{R277}} = 0.0 \text{ nm} \quad [7]$$

A sodium ion bound on a rotor site is offset from that site by 0.3 nm in the r direction. It has the coordinates

$$r_{\text{ion}} = 3.3 \text{ nm} ; \quad z_{\text{ion}} = 0.0 \text{ nm} \quad [8]$$

THE SODIUM F-ATPASE: SUPPLEMENTARY MATERIAL

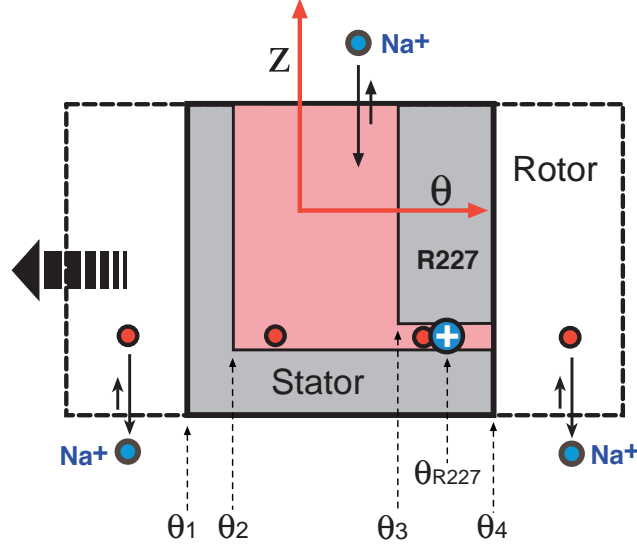


Fig. 13. The rotor-stator assembly projected onto the rotor surface, viewed from outside the rotor. Rotation during synthesis is to the left. If the stator spans three α -helices, then there are four rotor sites located near the stator: two within the rotor-stator interface and two adjacent laterally.

The electrostatic interaction between a charge (a negatively charged rotor binding site or a positively charged sodium ion) and the positive stator charge R227 is calculated using a shielded Coulomb's law. First the interaction according to Coulomb's law is calculated:

$$d(\theta, r, z) = \sqrt{[r_{R227} \cos(\theta_{R227}) - r \cos(\theta)]^2 + [r_{R227} \sin(\theta_{R227}) - r \sin(\theta)]^2 + z^2} \quad [9]$$

$$\tilde{\phi}(\theta, r, z) = \frac{e^2}{4\pi\epsilon_0} \cdot \frac{q}{\epsilon_s d} \cdot \exp(-\lambda d) = 56 k_B T \times \frac{q}{\epsilon d} \cdot \exp(-\lambda d)$$

In Eq. 9, (θ, r, z) is the cylindrical coordinate of the charge; $d(\theta, r, z)$ is the distance from the charge to the stator charge R227; q is the valence of the charge: $q = +1$ if the charge is positive (sodium ion) and $q = -1$ if the charge is negative (rotor binding site); ϵ_s is the dielectric constant of the stator; $1/\lambda$ is the Debye screening length. In our model, we take $\epsilon_s = 4$ and $1/\lambda = 1$ nm. However, when the charge is in the channel or outside the stator, it is surrounded by an aqueous environment with dielectric constant: $\epsilon_c \approx 80$. Once outside the stator, the interaction between the charge and R227 is much weaker. Thus we compute the interaction potential between the charge and R227 by modifying the Coulomb's law potential by:

$$\phi(\theta, r, z) = \begin{cases} \tilde{\phi}(\theta, r, z) - \tilde{\phi}(\theta_4, r, z) & \text{if } \theta_3 \leq \theta \leq \theta_4 \\ 0 & \text{otherwise} \end{cases} \quad [10]$$

The total electrostatic potential seen by the rotor has three parts:

THE SODIUM F-ATPASE: SUPPLEMENTARY MATERIAL

- (i) $\phi_Q(\theta, \mathbf{s})$ = the electrostatic interaction between all charges on the rotor and the positive stator charge R227.
- (ii) $\phi_{\Delta\psi}(\theta, \mathbf{s})$ = the interaction caused by the membrane potential drop.
- (iii) $\phi_{\Delta\epsilon}(\theta, \mathbf{s})$ = the interaction caused by the dielectric barrier that opposes the entry of a charged site into the hydrophobic rotor-stator interface.

$\phi_Q(\theta, \mathbf{s})$, is calculated by summing the contributions from the four rotor binding sites near the rotor-stator interface and the contributions from the sodium ions bound on them (if any).

At the level of the rotor binding sites, the potential due to the transmembrane potential drop is given by

$$\phi(\theta) = \begin{cases} \Delta\psi_{\text{mem}} \cdot (\theta - \theta_1) / (\theta_2 - \theta_1) & \text{if } \theta_1 \leq \theta \leq \theta_2 \\ \Delta\psi_{\text{mem}} & \text{if } \theta_2 \leq \theta \leq \theta_3 \\ \Delta\psi_{\text{mem}} \cdot (\theta_4 - \theta) / (\theta_4 - \theta_3) & \text{if } \theta_3 \leq \theta \leq \theta_4 \\ 0 & \text{otherwise} \end{cases} \quad [11]$$

$\phi_{\Delta\psi}(\theta, \mathbf{s})$ is calculated by summing the contributions from the charged (empty) rotor sites near the rotor-stator interface.

As shown in Fig. 13, the left boundary of the aqueous channel is formed by a rotor-stator interface with low dielectric constant. The effect on an uncharged (occupied) rotor site caused by this dielectric barrier is small; here we take it as zero. However, this dielectric barrier prevents a charged (empty) rotor site from entering the rotor-stator interface. The height of this barrier is given approximately by

$$\Delta\phi = 200 k_B T \times \left(\frac{1}{\epsilon_s} - \frac{1}{\epsilon_c} \right) \approx 45 k_B T \quad [12]$$

ϵ_c and ϵ_s are the dielectric constants of the cytoplasm and the stator, respectively (3). The potential caused by this dielectric barrier on a charged (empty) rotor site is

$$\phi(\theta) = \begin{cases} 45 k_B T & \text{if } \theta_1 \leq \theta \leq \theta_2 \\ 0 & \text{otherwise} \end{cases} \quad [13]$$

THE SODIUM F-ATPASE: SUPPLEMENTARY MATERIAL

The interaction potential caused by the dielectric barrier, $\phi_{\Delta\epsilon}(\theta, \mathbf{s})$, is calculated by summing up the contributions from the charged (empty) rotor sites near the rotor-stator interface.

Langevin Equation and Fokker-Planck Equation

The motion of the rotor can be described by equating the viscous drag on the rotor to the torques that act on the rotor and the Brownian force modeling the rotor's thermal fluctuations [i.e. the Langevin equation (4)]:

$$\underbrace{\zeta \frac{d\theta}{dt}}_{\text{Frictional drag}} = \underbrace{\tau_{\varrho}(\theta, \mathbf{s})}_{\text{Rotor - stator charge interaction}} + \underbrace{\tau_{\Delta\psi}(\theta, \mathbf{s})}_{\text{Membrane potential}} + \underbrace{\tau_{\Delta\epsilon}(\theta, \mathbf{s})}_{\text{Dielectric barrier}} + \underbrace{\tau_{\text{RS}}(\theta)}_{\text{Rotor - stator passive interaction}} - \underbrace{\tau_{\text{L}}(\theta)}_{\text{Load torque from } F_1} + \underbrace{\tau_{\text{B}}(t)}_{\text{Brownian torque}}, \quad \mathbf{s} = 1, \dots, 16 \quad [14]$$

Chemical states

where

$$\begin{aligned} \tau_{\varrho}(\theta, \mathbf{s}) &= -\frac{\partial \phi_{\varrho}(\theta, \mathbf{s})}{\partial \theta} \\ \tau_{\Delta\psi}(\theta, \mathbf{s}) &= -\frac{\partial \phi_{\Delta\psi}(\theta, \mathbf{s})}{\partial \theta} \\ \tau_{\Delta\epsilon}(\theta, \mathbf{s}) &= -\frac{\partial \phi_{\Delta\epsilon}(\theta, \mathbf{s})}{\partial \theta} \\ \tau_{\text{RS}}(\theta) &= -\frac{\partial \phi_{\text{RS}}(\theta)}{\partial \theta} \end{aligned} \quad [15]$$

As discussed above, the three electrostatic torques depend on the chemical state of the rotor site, \mathbf{s} ; that is, whether the site is charged (unoccupied) or uncharged (occupied). For each chemical state, \mathbf{s} , the sum of the first four terms in Eq. 14 can be expressed as the derivative of a potential function:

$$\tau(\theta, \mathbf{s}) = -\frac{\partial}{\partial \theta} \Phi(\theta, \mathbf{s}) \equiv -\frac{\partial}{\partial \theta} (\phi_{\varrho} + \phi_{\Delta\psi} + \phi_{\Delta\epsilon} + \phi_{\text{RS}}) \quad [16]$$

Thus Eq. 14 can be written compactly as

$$\zeta \frac{d\theta}{dt} = -\frac{\partial \Phi(\theta, \mathbf{s})}{\partial \theta} - \tau_{\text{L}}(\theta) + \tau_{\text{B}}(t), \quad \mathbf{s} = 1, \dots, 16 \quad [17]$$

In Eq. 17, ζ is the rotational drag coefficient, θ is the angular coordinate, $\Phi(\theta, \mathbf{s})$ is the sum of the interaction potentials between the rotor and the stator, τ_{L} is the

THE SODIUM F-ATPASE: SUPPLEMENTARY MATERIAL

load torque, and τ_B is the Brownian torque due to thermal fluctuations. \mathbf{s} represents the binding status of the four rotor sites near the rotor-stator interface. \mathbf{s} can take any of the $2^4 = 16$ possible states on the chemical torus shown in Fig. 12. The probability of state \mathbf{s} evolves according to the kinetic equation 2, which we rewrite in the paper symbolically as

$$\frac{d}{dt} \mathbf{s} = \mathbf{K}(\theta) \cdot \mathbf{s} \quad [18]$$

To compute the stochastic behavior of the F_o motor (a sequence of the rotor positions corresponding to different times), Eq. 17 must be solved simultaneously with the Markov process governing the kinetic transitions on the rotor binding sites (Eq. 18).

For the purpose of computing statistical averages of the motion, such as the load-velocity behavior of the motor, one can recast the model as an equivalent convection diffusion (Fokker-Planck) equation governing the evolution of the probability density (4). In the Fokker-Planck formulation corresponding to Eqs. 17 and 18, the F_o motor is described by a vector function consisting of 16 probability density functions, one for each chemical state. Let $\rho(\theta, t, \underline{k})$ be the probability density that the rotor is at the angular position θ at time t , and the system is in k th state. Define the probability density vector as:

$$\rho(\theta, t) = \left[\begin{array}{c} \rho(\theta, t, 1) \\ \rho(\theta, t, 2) \\ \vdots \\ \rho(\theta, t, 15) \\ \rho(\theta, t, 16) \end{array} \right] \left. \vphantom{\begin{array}{c} \rho(\theta, t, 1) \\ \rho(\theta, t, 2) \\ \vdots \\ \rho(\theta, t, 15) \\ \rho(\theta, t, 16) \end{array}} \right\} \text{16 components} \quad [19]$$

This probability density vector evolves according to the convection diffusion equations

$$\frac{\partial \rho}{\partial t} = \underbrace{\frac{1}{\zeta} \frac{\partial}{\partial \theta} (\Phi' \rho)}_{\text{Forces between rotor and stator}} + \underbrace{\frac{1}{\zeta} \frac{\partial}{\partial \theta} (\tau_L(\theta) \rho)}_{\text{Load torque}} + \underbrace{D \frac{\partial^2 \rho}{\partial \theta^2}}_{\text{Brownian motion}} + \underbrace{\mathbf{K}(\theta) \rho}_{\text{Transitions between rotor states}} \quad [20]$$

Here $D = k_B T / \zeta$ is the rotor diffusion coefficient. The 16×16 transition matrix $\mathbf{K}(\theta)$ is defined in Eq. 3. The potential matrix $\Phi(\theta)$ is diagonal and is given by

THE SODIUM F-ATPASE: SUPPLEMENTARY MATERIAL

$$\frac{d}{dt} \begin{bmatrix} \rho(t, \theta; 1) \\ \rho(t, \theta; 2) \\ \vdots \\ \rho(t, \theta; 80) \\ \rho(t, \theta; 81) \end{bmatrix} = \mathbf{K}(\theta) \cdot \begin{bmatrix} \rho(t, \theta; 1) \\ \rho(t, \theta; 2) \\ \vdots \\ \rho(t, \theta; 80) \\ \rho(t, \theta; 81) \end{bmatrix} \left. \vphantom{\begin{bmatrix} \rho(t, \theta; 1) \\ \rho(t, \theta; 2) \\ \vdots \\ \rho(t, \theta; 80) \\ \rho(t, \theta; 81) \end{bmatrix}} \right\} \text{81 components} \quad [22]$$

For each chemical state, the interaction between the rotor and the stator is calculated using the procedures described above. The calculation of this interaction does not depend on whether the bound sodium ion is labeled. The Fokker-Planck equation for the system has the same form as Eq. 20 except that it now has 81 components instead of 16. Treating $^{22}\text{Na}^+$ and Na^+ as two kinds of ions enables us to calculate the flux of $^{22}\text{Na}^+$ and the flux of Na^+ separately, from which, the initial $^{22}\text{Na}^+$ uptake rate is computed.

Simulating the Behavior of the F₀ Pump

A torque generated in F₁ from ATP hydrolysis can drive the F₀ motor in reverse turning it into an ion pump. To simulate this behavior, we coupled the mathematical equations for the F₀ motor in this paper to the mathematical equations for the F₁ motor formulated by Wang and Oster (5) subject to the following constraints:

- The F₀ motor and the F₁ motor rotate with the same velocity.
- The load torque on the F₁ motor (i.e., the torque that the F₁ motor is working against) is the same as the driving torque on the F₀ motor.

The resulting ion flux could then be computed by solving the coupled system.

References

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