

Mathematical formulation of the F_0 motor model

A. Markov chain model for the evolution of the rotor state

The four possible protonation states of the two rotor Asp61 sites at the rotor-stator interface are:

Both sites empty (E) :	(o o)
The right site protonated (R) :	(o •)
Both sites protonated (F) :	(• •)
The left site protonated (L) :	(• o)

As protons hop into and out of the channel, the state of the two exposed rotor sites changes. Figure 1 shows the transitions between these rotor states due to proton hops. The Markov transition matrix corresponding to the transition diagram in Figure 1 is:

$$\mathbf{K} = \begin{bmatrix} -(k_{ER} + k_{EL}) & k_{RE} & 0 & k_{LE} \\ k_{ER} & -(k_{RE} + k_{RF}) & k_{FR} & 0 \\ 0 & k_{RF} & -(k_{FR} + k_{FL}) & k_{LF} \\ k_{EL} & 0 & k_{FL} & -(k_{LE} + k_{LF}) \end{bmatrix} \quad (1)$$

We assume that protons cannot hop between the Asp61 sites. At any fixed rotor position, it takes two proton hops to go from state **R** (o •) to state **L** (• o) (i.e. one proton hops out and another one hops in). So there is no direct Markov transition between **R** and **L** in Figure 1. However the transition between **R** and **L** can be done by a rotation of the rotor (shown as dashed line in Figure 1). For example, when the rotor is in state **R** (o •), a rotation to the right can push the protonated site on the right into the membrane and pull a protonated site out of the membrane on the left, which changes the rotor into state **L** (• o).

In Figure 2, we plot the free energy change, $\Delta G(\theta, \mathbf{s})$, in the system as a function of rotation angle, θ , and rotor state, \mathbf{s} , when one proton passes through the motor producing a rotation of $2\pi/12$ radian against a load torque of 41 pN-nm. The cycle shown is the power cycle **E** \rightarrow **R** \rightarrow **L** \rightarrow **E**. The protonmotive force across the membrane is $\Delta p = 220$ mV ($= 8.8 k_B T$). The work done by the proton against the load torque is $41 \text{ pN-nm} \times (2\pi/12) = 5.2 k_B T$. The energy dissipated in the process is $8.8 - 5.2 = 3.6 k_B T$. In Figure 2, the free energy curve for state **E** at the end of the cycle is $3.6 k_B T$ lower than the one for state **E** at the beginning of the cycle.

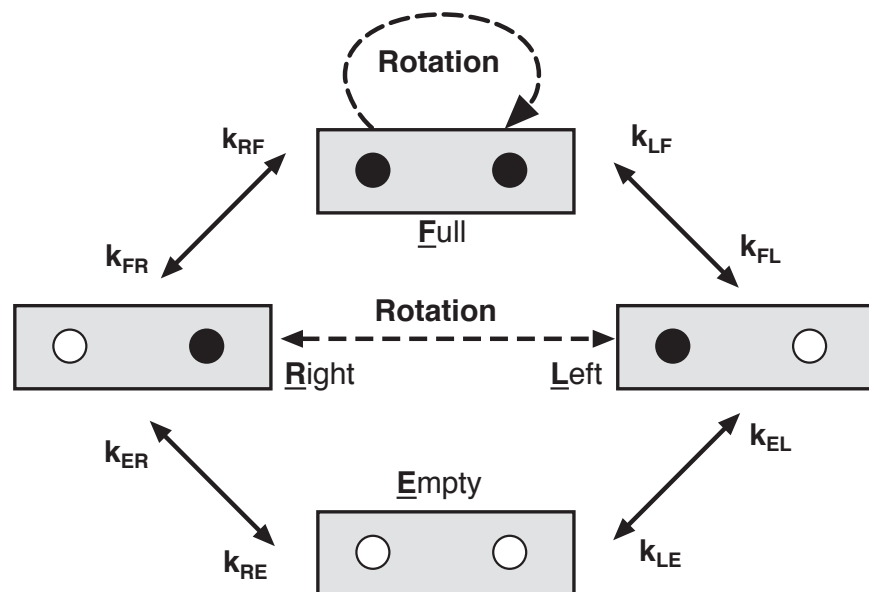


Figure 1 Markov chain describing the four possible rotor states and their transition rates, $k_{i \rightarrow j}$. E = Empty, F = Full, L = Left site occupied, R = Right site occupied. (o) = unprotonated Asp61 sites, (•) = protonated sites.

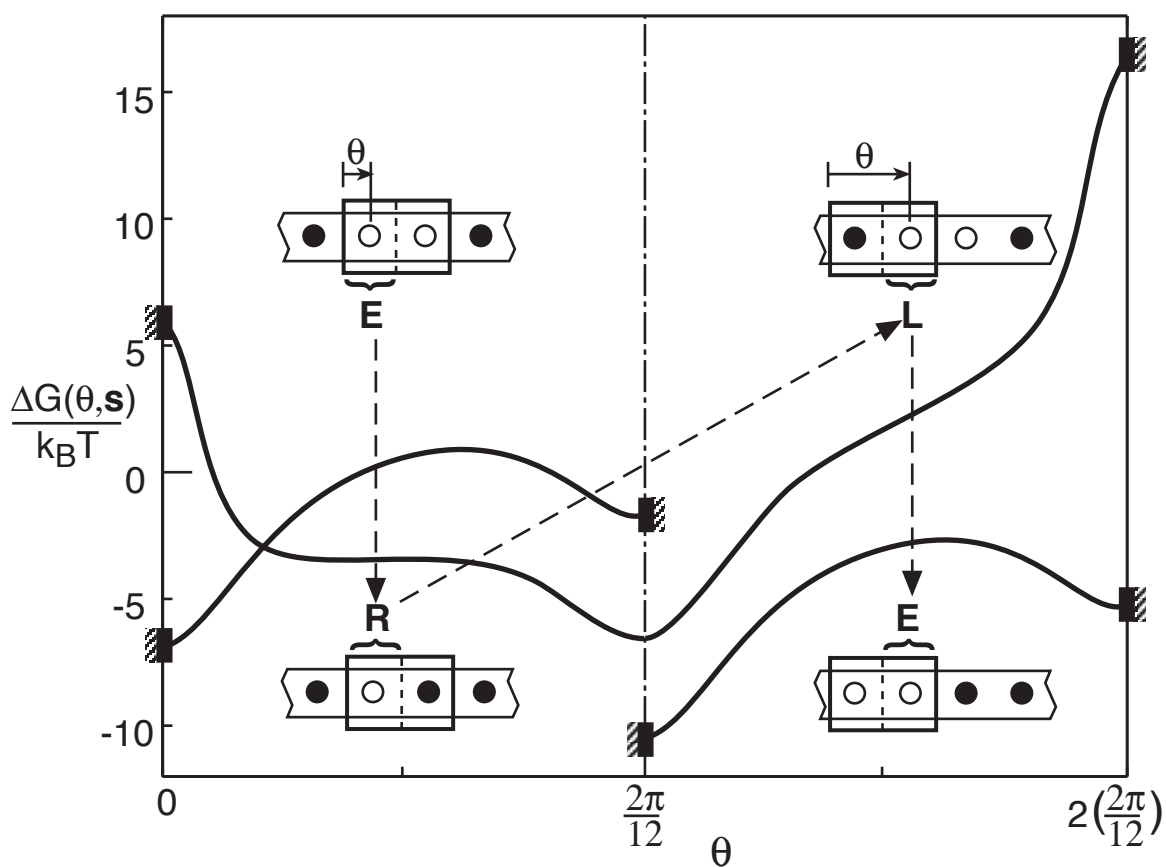


Figure 2 Free energy change, $\Delta G(\theta, \mathbf{s})$, in the system as a function of rotation angle, θ , and rotor state, \mathbf{s} , when one proton passes through the motor producing a rotation of $2\pi/12$ radian against a load torque of 41 pN-nm. The reflecting membrane boundaries are denoted by solid rectangles. The cycle shown is the power cycle $\mathbf{E} \rightarrow \mathbf{R} \rightarrow \mathbf{L} \rightarrow \mathbf{E}$; other cycles shown in Figure 1 are not power producing, and involve rotor slip and/or proton leakage. The protonmotive force across the membrane is $\Delta p = 220 \text{ mV} \approx 8.8 k_B T$. The work done by the proton against the load torque is $41 \text{ pN-nm} \times (2\pi/12) = 5.2 k_B T$. The energy dissipated in the process is $8.8 - 5.2 = 3.6 k_B T$, so the free energy curve for state \mathbf{E} at the end of the cycle is $3.6 k_B T$ lower than the one for state \mathbf{E} at the beginning of the cycle.

B. Langevin equation formulation

Since inertia is negligible, equating the viscous drag on the rotor to the forces acting on it yields the Langevin equation ^{1, 2}

$$\zeta \frac{d\theta}{dt} = -\frac{d\psi_E(\theta; \mathbf{s})}{d\theta} - \frac{d\phi_H(\theta; \mathbf{s})}{d\theta} - \tau + f(t), \quad \mathbf{s} = \mathbf{L}, \mathbf{R}, \mathbf{E}, \mathbf{F} \quad (2)$$

where:

- ζ = drag coefficient of the c-rotor.
- $f(t)$ = the random force due to brownian fluctuations. The brownian force is modeled in the usual way by a Gaussian distribution with unit mean and amplitude $\sqrt{2\zeta k_B T \Delta t}$, where Δt is the time step in the numerical simulation ².
- τ = load torque from F_1 .
- $\mathbf{s} = (\mathbf{E}, \mathbf{R}, \mathbf{F}, \mathbf{L})$. The state of the two exposed rotor sites evolves according to the transition rates k_{ij} shown in Figure 1.
- $\psi_E(\theta; \mathbf{s})$ = the potential affecting the rotor in state \mathbf{s} . $\psi_E(\theta; \mathbf{s})$ contains the interactions between the rotor sites and the fixed stator charges (Arg210, Glu219, and His245).
- $\phi_H(\theta; \mathbf{s})$ = the hydrophobic potential barrier preventing the rotation of unprotonated rotor sites into the membrane. The energy barrier against rotating an unprotonated Asp61 site into the bilayer depends on the difference in the dielectric constants between the two media: $\Delta G \approx 200 \left(\frac{1}{\epsilon_{\text{membrane}}} - \frac{1}{\epsilon_{\text{stator}}} \right) 3$. Taking $\epsilon_{\text{mem}} \approx 3$ and $\epsilon_{\text{stator}} \approx 10$, we have $\Delta G \approx 45 k_B T$.

Note that $\psi_E(\theta; \mathbf{s})$ and $\phi_H(\theta; \mathbf{s})$ could be combined into one potential in equation (2).

However, we keep them separate because in the Fokker-Planck formulation, the effect of the membrane potential barrier, $\phi_H(\theta; \mathbf{s})$, is modeled by boundary conditions, while

the effect of rotor-stator charge interactions are carried by the potential $\psi(\theta; \mathbf{s})$, which resides in the equations of motion.

To compute the torque generated by the motor, equations (2) must be solved simultaneously with the Markov process governing the hopping of protons on and off of the rotor sites. Although we draw the rotation of the rotor as a link (dashed line) between states **R** and **L**, the rotation of the rotor from θ to $\theta+2\pi/12$ cannot be treated simply as a single Markov step for the reasons listed below:

- The rotation of the rotor is continuous. The rotation of the rotor from θ to $\theta+\delta$ can be treated as a single Markov step only if the diffusion of the rotor in $[\theta, \theta+\delta]$ is much faster than the net rotation of the rotor from θ to $\theta+\delta$. The time scale for diffusion in $[\theta, \theta+\delta]$ is $\sim\delta^2/D$ and the time scale of the net rotation is $\sim\delta/\langle v \rangle$. Therefore, if δ is small enough, the diffusion is always faster than the net rotation. This is the essence of the numerical discretization of the model. However, $\delta = 2\pi/12$ is not small enough, so the rotation from θ to $\theta+2\pi/12$ cannot be treated as a single Markov step; instead it has to be treated as a sequence of smaller Markov steps.
- The effect of the stator charge, Arg210, on the rotor is strongly angle-dependent. When a protonated rotor site passes Arg210, the stator charge reduces the effective pK_a of the rotor site and forces the rotor site to relinquish its proton. When a rotor site is far from the stator charge, its large pK_a can hold the proton tightly. This prevents many futile protonation and deprotonation cycles. If we treat the rotation from θ to $\theta+2\pi/12$ as a single Markov step, then we cannot model the angle-dependence of the interactions between the stator and rotor charges.

C. Fokker-Planck equation formulation

In the Fokker-Planck formulation corresponding to equations (2), the state of the system is described by the four probability density functions:

$$\boldsymbol{\rho}(\theta, t) = \begin{bmatrix} \rho_E(\theta, t) \\ \rho_R(\theta, t) \\ \rho_F(\theta, t) \\ \rho_L(\theta, t) \end{bmatrix} \quad (3)$$

where $\rho_s(\theta, t)$ is the probability density that the motor is in state **s** and the rotor is at location θ at time t . These distributions evolve according to the convective diffusion equations

$$\frac{\partial \boldsymbol{\rho}}{\partial t} = \frac{1}{\zeta_r} \frac{\partial}{\partial \theta} \left\{ \left(\frac{d\Psi}{d\theta} + \boldsymbol{\tau} \right) \boldsymbol{\rho} \right\} + D_r \frac{\partial^2 \boldsymbol{\rho}}{\partial \theta^2} + \mathbf{K} \boldsymbol{\rho} \quad (4)$$

where $D_r = k_B T / \zeta_r$ is the diffusion coefficient of the c-rotor. The electrostatic potential matrix is

$$\Psi(\theta) = \begin{bmatrix} \psi_E(\theta) & 0 & 0 & 0 \\ 0 & \psi_R(\theta) & 0 & 0 \\ 0 & 0 & \psi_F(\theta) & 0 \\ 0 & 0 & 0 & \psi_L(\theta) \end{bmatrix} \quad (5)$$

where $\psi_s(\theta)$ is the potential acting on the rotor due to the electrostatic interactions between the rotor sites and the stator charges when the two rotor sites are in state s .

The boundary conditions for equation (4) are given by

- Boundary condition for ρ_E ((o o): reflecting at $\theta = 0$ and at $\delta = 2\pi/12$):

$$\left\{ \frac{1}{\zeta} \left(\frac{d\psi_E}{d\theta} + \tau \right) \rho_E + D \frac{\partial \rho_E}{\partial \theta} \right\}_{\theta=0} = 0; \quad \left\{ \frac{1}{\zeta} \left(\frac{d\psi_E}{d\theta} + \tau \right) \rho_E + D \frac{\partial \rho_E}{\partial \theta} \right\}_{\theta=\delta} = 0 \quad (6)$$

- Boundary condition for ρ_R ((o •): reflecting at $\theta = 0$):

$$\left\{ \frac{1}{\zeta} \left(\frac{d\psi_R}{d\theta} + \tau \right) \rho_R + D \frac{\partial \rho_R}{\partial \theta} \right\}_{\theta=0} = 0 \quad (7)$$

- Boundary condition for ρ_F ((• •): periodic with period = δ):

$$\left\{ \frac{1}{\zeta} \left(\frac{d\psi_F}{d\theta} + \tau \right) \rho_F + D \frac{\partial \rho_F}{\partial \theta} \right\}_{\theta=0} = \left\{ \frac{1}{\zeta} \left(\frac{d\psi_F}{d\theta} + \tau \right) \rho_F + D \frac{\partial \rho_F}{\partial \theta} \right\}_{\theta=\delta} \quad (8)$$

- Boundary condition for ρ_L ((• o): reflecting at $\theta = \delta$):

$$\left\{ \frac{1}{\zeta} \left(\frac{d\psi_L}{d\theta} + \tau \right) \rho_L + D \frac{\partial \rho_L}{\partial \theta} \right\}_{\theta=\delta} = 0 \quad (9)$$

- The probability flux leaving the right end ($\theta = \delta$) of state **R** is equal to the flux entering the left end ($\theta = 0$) of state **L**:

$$\left\{ \frac{1}{\zeta} \left(\frac{d\psi_R}{d\theta} + \tau \right) \rho_R + D \frac{\partial \rho_R}{\partial \theta} \right\}_{\theta=\delta} = \left\{ \frac{1}{\zeta} \left(\frac{d\psi_L}{d\theta} + \tau \right) \rho_L + D \frac{\partial \rho_L}{\partial \theta} \right\}_{\theta=0} \quad (10)$$

D. Numerical Calculations

D.1 Motor without Arg210: A pure Brownian ratchet

In this model, we assume:

- k_{in}^A = the rate of proton hopping into the channel from the acidic side (low pH) of the membrane. It is independent of the rotor position, θ , and independent of the state of the other site.

- k_{in}^B = the rate of proton hopping into the channel from the basic side (high pH) of the membrane. It is independent of the rotor position, θ , and independent of the state of the other site.
- k_{out}^A = the rate of proton hopping off the site to the acidic side. It is independent of the rotor position, θ , and independent of the state of the other site.
- k_{out}^B = the rate of proton hopping off the site to the basic side. It is independent of the rotor position, θ , and independent of the state of the other site.

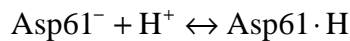
These transition rates depend on the parameters listed below:

- pH^A = proton concentration on the acidic side (low pH) of the membrane.
- pH^B = proton concentration on the basic side (high pH) of the membrane.
- $\Delta\psi$ = potential drop across the membrane.
- $\Delta\phi^A$ = the potential drop at surface (due to surface charges) at the acidic side of the membrane.
- $\Delta\phi^B$ = the potential drop at surface (due to surface charges) at the basic side of the membrane.
- D_p = proton diffusion coefficient.
- r = radius of the channel leading to the rotor sites.

D.2 Transition rates for ratchet model

In this subsection we first use k_{in}^A and k_{out}^A as an example to demonstrate our step-by-step procedure for calculating the transition rates. Then we give the rate formulas for the model without Arg210.

The reaction on the rotor sites is represented by



At equilibrium,

$$k_{in} \cdot [\text{Asp61}^-] = k_{out} \cdot [\text{Asp61} \cdot \text{H}] \quad (11)$$

By definition, $pK_a = pH + \log_{10} \frac{[\text{Asp61} \cdot \text{H}]}{[\text{Asp61}^-]}$, from which

$$\frac{[\text{Asp61} \cdot \text{H}]}{[\text{Asp61}^-]} = 10^{pK_a - pH} \quad (12)$$

The proton hopping rates, k_{in} and k_{out} , are related to pK_a by combining (11) and (12):

$$\frac{k_{in}}{k_{out}} = 10^{pK_a - pH}$$

First consider the case where the potential drop across the membrane is zero and there is no surface charge on the membrane. For the acidic reservoir we have

$$\frac{k_{in}^A(0)}{k_{out}^A(0)} = 10^{pK_a - pH^A} \quad (13)$$

The rate $k_{in}^A(0)$ is limited by the rate protons can jump into the channel. We compute the proton rate constants for entry into the channels by the Smoluchowski formula ⁴:

$$k_{in} = \left(\frac{\text{surface proton}}{\text{concentration}} \right) \cdot \left(\frac{\text{absorption rate to a perfectly}}{\text{absorbing disk of radius } r} \right) \quad (14)$$

The rate $k_{in}^A(0)$ is given by♦

$$k_{in}^A(0) = \underbrace{0.6 \text{ nm}^{-3} \cdot 10^{-pH^A}}_{\text{proton concentration at the acidic side}} \cdot \underbrace{4r \cdot D_p}_{\text{absorption rate}} \quad (15)$$

From equation (13), the rate $k_{out}^A(0)$ is given by

$$k_{out}^A(0) = 0.6 \text{ nm}^{-3} \cdot 10^{-pK_a} \cdot 4r \cdot D_p \quad (16)$$

Using the parameter values listed in **Table 1** ($pH^A = 7$, $r = 0.5 \text{ nm}$ and $D_p = 9.3 \times 10^9 \text{ nm}^2/\text{s}$), we have $k_{in}^A(0) = 1.1 \times 10^3/\text{s}$. This is the rate of protons hopping into the channel from the acidic side when no surface charge is on the membrane. This proton in-rate is too small to achieve the ATP synthesis rate of 400 ATP/s. The proton in-rate can be increased by increasing the proton concentration near the membrane surface.

The surface charges on the membrane increase (or decrease) the surface concentration of protons, and change the potential drop inside the membrane. The potential drop inside the membrane is the sum of the contribution from membrane potential and the contribution from the surface charges on the membrane. The potential drop inside the membrane is

$$\Delta\psi - (\Delta\phi^A - \Delta\phi^B) \quad (17)$$

If there is equal amount of surface charges on each side (i.e., $\Delta\phi^A = \Delta\phi^B$), the contribution of the surface charges is zero. The potential drop inside the membrane does not affect the rate of protons jumping into the channel. It only changes the rate

♦ 10^{-pH} is the proton concentration in mole/liter. 1 mole/liter = 0.6 molec / nm^3

of protons jumping off the site. So the in-rate and the off-rate for the model without Arg210 are given by

$$k_{\text{in}}^{\text{A}} = 0.6 \text{ nm}^{-3} \cdot 10^{-\text{pH}^{\text{A}}} \cdot 4r \cdot D_{\text{p}} \cdot \exp\left(\frac{\Delta\phi^{\text{A}}}{k_{\text{B}}T}\right) \quad (18)$$

$$k_{\text{out}}^{\text{A}} = 0.6 \text{ nm}^{-3} \cdot 10^{-\text{pK}_{\text{a}}} \cdot 4r \cdot D_{\text{p}} \cdot \exp\left(\frac{(\Delta\phi^{\text{A}} - \Delta\phi^{\text{B}}) - \Delta\psi}{2k_{\text{B}}T}\right) \quad (19)$$

$$k_{\text{in}}^{\text{B}} = 0.6 \text{ nm}^{-3} \cdot 10^{-\text{pH}^{\text{B}}} \cdot 4r \cdot D_{\text{p}} \cdot \exp\left(\frac{\Delta\phi^{\text{B}}}{k_{\text{B}}T}\right) \quad (20)$$

$$k_{\text{out}}^{\text{B}} = 0.6 \text{ nm}^{-3} \cdot 10^{-\text{pK}_{\text{a}}} \cdot 4r \cdot D_{\text{p}} \cdot \exp\left(\frac{\Delta\psi - (\Delta\phi^{\text{A}} - \Delta\phi^{\text{B}})}{2k_{\text{B}}T}\right) \quad (21)$$

For the model without Arg210, the transition matrix entries are given by

$$\begin{aligned} k_{\text{ER}} &= k_{\text{LF}} = k_{\text{in}}^{\text{A}} \\ k_{\text{RE}} &= k_{\text{FL}} = k_{\text{out}}^{\text{A}} \\ k_{\text{EL}} &= k_{\text{RF}} = k_{\text{in}}^{\text{B}} \\ k_{\text{LE}} &= k_{\text{FR}} = k_{\text{out}}^{\text{B}} \end{aligned} \quad (22)$$

D.3 Motor including Arg210: Electrostatically assisted ratchet

The transition rates for the model with Arg210 are given by

$$k_{\text{ER}}(\theta) = 0.6 \text{ nm}^{-3} \cdot 10^{-\text{pH}^{\text{A}}} \cdot 4r \cdot D_{\text{p}} \cdot \exp\left(\frac{\Delta\phi^{\text{A}}}{k_{\text{B}}T}\right) \quad (23)$$

$$k_{\text{RE}}(\theta) = 0.6 \text{ nm}^{-3} \cdot 10^{-\text{pK}_{\text{a}}} \cdot 4r \cdot D_{\text{p}} \cdot \exp\left(\frac{(\Delta\phi^{\text{A}} - \Delta\phi^{\text{B}}) - \Delta\psi}{2k_{\text{B}}T}\right) \cdot \exp\left(\frac{\psi_{\text{R}}(\theta) - \psi_{\text{E}}(\theta)}{k_{\text{B}}T}\right) \quad (24)$$

$$k_{\text{EL}}(\theta) = 0.6 \text{ nm}^{-3} \cdot 10^{-\text{pH}^{\text{B}}} \cdot 4r \cdot D_{\text{p}} \cdot \exp\left(\frac{\Delta\phi^{\text{B}}}{k_{\text{B}}T}\right) \quad (25)$$

$$k_{\text{LE}}(\theta) = 0.6 \text{ nm}^{-3} \cdot 10^{-\text{pK}_{\text{a}}} \cdot 4r \cdot D_{\text{p}} \cdot \exp\left(\frac{\Delta\psi - (\Delta\phi^{\text{A}} - \Delta\phi^{\text{B}})}{2k_{\text{B}}T}\right) \cdot \exp\left(\frac{\psi_{\text{L}}(\theta) - \psi_{\text{E}}(\theta)}{k_{\text{B}}T}\right) \quad (26)$$

$$k_{\text{LF}}(\theta) = 0.6 \text{ nm}^{-3} \cdot 10^{-\text{pH}^{\text{A}}} \cdot 4r \cdot D_{\text{p}} \cdot \exp\left(\frac{\Delta\phi^{\text{A}}}{k_{\text{B}}T}\right) \quad (27)$$

$$k_{\text{FL}}(\theta) = 0.6 \text{ nm}^{-3} \cdot 10^{-\text{pK}_{\text{a}}} \cdot 4r \cdot D_{\text{p}} \cdot \exp\left(\frac{(\Delta\phi^{\text{A}} - \Delta\phi^{\text{B}}) - \Delta\psi}{2k_{\text{B}}T}\right) \cdot \exp\left(\frac{\psi_{\text{F}}(\theta) - \psi_{\text{L}}(\theta)}{k_{\text{B}}T}\right) \quad (28)$$

$$k_{\text{RF}}(\theta) = 0.6 \text{ nm}^{-3} \cdot 10^{-\text{pH}^{\text{B}}} \cdot 4r \cdot D_p \cdot \exp\left(\frac{\Delta\phi^{\text{B}}}{k_{\text{B}}T}\right) \quad (29)$$

$$k_{\text{FR}}(\theta) = 0.6 \text{ nm}^{-3} \cdot 10^{-\text{pK}_a} \cdot 4r \cdot D_p \cdot \exp\left(\frac{\Delta\psi - (\Delta\phi^{\text{A}} - \Delta\phi^{\text{B}})}{2k_{\text{B}}T}\right) \cdot \exp\left(\frac{\psi_{\text{F}}(\theta) - \psi_{\text{R}}(\theta)}{k_{\text{B}}T}\right) \quad (30)$$

E. The electrostatic forces act against the rotor motion

The average torque generated directly by the electrostatic interactions between the rotor and stator can be computed from

$$\langle \tau_{\text{Electrostatic}} \rangle = \sum_{\text{S=E,L,R,F}} \int_0^{\frac{2\pi}{12}} \rho_{\text{S}}(\theta) \cdot [-\psi'_{\text{S}}(\theta)] d\theta \quad (31)$$

At the operating point for ATP synthesis, the average torque generated directly by the electrostatic interactions between the rotor and stator is negative. However this is more than compensated by the effect of the electrostatic interactions on the rotor sites' pK_a 's, which tightly couples the proton flux to the rotor motion, and increase the effectiveness of rectifying the rotor's diffusion.

Table 1 lists the parameter values used in the numerical simulations.

The complete simulation code in Matlab™ 5 is available on request.

PARAMETER	Value	
D_p = proton diffusion coefficient	9.3×10 ⁹ nm ² /s	
D_r = rotary diffusion coefficient of the rotor	2×10 ⁴ /s *	
ϵ_c = dielectric constant of channel	10	
ϵ_m = dielectric constant of the membrane	3	
η = bilayer viscosity	1 poise	
h = height of the rotor	6 nm	
$1/\lambda$ = shielding length of stator charges	1.1 nm	
pH^A = bulk pH of the acidic reservoir	motor = 7	pump = 6.6
pH^B = bulk pH of the basic reservoir	motor = 8.4	pump = 7.6
r = 'radius' of the proton channel	0.5 nm	
R = radius of rotor	5 nm	
Δx = distance between Asp61 residues = $2\pi R/12$	2.6 nm	
ΔpH = pH difference across the membrane	80 mV = 3.2 $k_B T$	
$\Delta \psi$ = membrane potential	140 mV = 5.6 $k_B T$	
$\Delta \phi^A$ = potential drop by surface charges at the acidic side	2.3 $k_B T$ (without Arg210) ♦ 2.0 $k_B T$ (with Arg210)	
$\Delta \phi^B$ = potential drop by surface charges at the basic side	2.3 $k_B T$ (without Arg210) ♦ 2.0 $k_B T$ (with Arg210)	

Table 1. Parameter values.

* The diffusion coefficient of the rotor was computed from $D_r = k_B T / (6\pi\eta h R^2)$, and was modified to reflect the fact that part of the rotor is not subject to the membrane viscosity.

♦ Potential drop of 2.3 $k_B T$ by surface charges can reduce the surface pH value (increase the surface concentration) by 1.

References

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