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Supplementary Information for

Torque-dependent remodeling of the bacterial flagellar motor

Navish Wadhwa, Rob Phillips and Howard C. Berg

Navish Wadhwa
E-mail: navish_wadhwa@fas.harvard.edu

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Supplementary text
Figs. S1 to S4

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Supporting Information Text

A. Derivation of the kinetics equation from the chemical master equation. The instantaneous probability distribution for the number of stator units bound to the motor is governed by the chemical master equation

$$\frac{\mathrm{d}p(n,t)}{\mathrm{d}t} = (n+1)k_{-}p(n+1,t) + (N-n+1)k_{+}p(n-1,t) - nk_{-}p(n,t) - (N-n)k_{+}p(n,t) \qquad [1]$$

Each term in Eq. 1 accounts for a transition that either brings the number of bound units to n or moves it away from n. Probability of gaining a unit is proportional to the number of empty binding sites, therefore the two gain terms contain $(N-n+1)$ and $(N-n)$ respectively. Similarly, the probability of losing a unit is proportional to the number of bound units, hence the two loss terms contain $(n+1)$ and n, respectively. To go from the chemical master equation to a kinetics equation for the average number of bound units, multiply both sides by n and sum over all possible values of n, i.e. from 0 to N,

$$\begin{aligned}
\sum_{n=0}^{N} n\frac{\mathrm{d}p(n,t)}{\mathrm{d}t} &= \sum_{n=0}^{N} n(n+1)k_{-}p(n+1,t) + \sum_{n=0}^{N} n(N-n+1)k_{+}p(n-1,t) - \sum_{n=0}^{N} n^2k_{-}p(n,t) - \sum_{n=0}^{N} n(N-n)k_{+}p(n,t) \\
\frac{\mathrm{d}}{\mathrm{d}t}\sum_{n=0}^{N} np(n,t) &= k_{+}\left(\sum_{n=0}^{N} n(N-n+1)p(n-1,t) - \sum_{n=0}^{N} n(N-n)p(n,t)\right) + k_{-}\left(\sum_{n=0}^{N} n(n+1)p(n+1,t) - \sum_{n=0}^{N} n^2p(n,t)\right).
\end{aligned}$$

$\sum_{n=0}^{N} np(n,t) = \langle n\rangle$ and we can make index substitutions to convert all probability terms to $p(n,t)$. Knowing that $p(n) = 0$ for $n < 0$ or $n > N$, we get

$$\begin{aligned}
\frac{\mathrm{d}\langle n\rangle}{\mathrm{d}t} &= k_{+}\left(\sum_{n=0}^{N-1}(n+1)(N-n)p(n,t) - \sum_{n=0}^{N} n(N-n)p(n,t)\right) + k_{-}\left(\sum_{n=1}^{N} n(n-1)p(n,t) - \sum_{n=0}^{N} n^2p(n,t)\right) \\
&= k_{+}\left(\sum_{n=0}^{N}(n+1)(N-n)p(n,t) - \sum_{n=0}^{N} n(N-n)p(n,t)\right) + k_{-}\left(\sum_{n=0}^{N} n(n-1)p(n,t) - \sum_{n=0}^{N} n^2p(n,t)\right),
\end{aligned}$$

since $(n+1)(N-n)p(n,t) = 0$ at $n = N$ and $n(n-1)p(n,t) = 0$ at $n = 0$. The different terms can now be combined, to give

$$\begin{aligned}
\frac{\mathrm{d}\langle n\rangle}{\mathrm{d}t} &= k_{+}\left(\sum_{n=0}^{N}(n+1-n)(N-n)p(n,t)\right) + k_{-}\left(\sum_{n=0}^{N}(n^2-n-n^2)p(n,t)\right) \\
&= k_{+}\left(N\sum_{n=0}^{N} p(n,t) - \sum_{n=0}^{N} np(n,t)\right) - k_{-}\left(\sum_{n=0}^{N} np(n,t)\right) \\
&= k_{+}(N-\langle n\rangle) - k_{-}\langle n\rangle
\end{aligned}$$

which is the kinetics equation for the average number of stator units as a function of time. The steady state solution for this equation can be found by setting the rate of change $\frac{\mathrm{d}\langle n\rangle}{\mathrm{d}t}$ equal to 0, such that

$$\begin{aligned}
k_{+}(N-\langle n\rangle_{\mathrm{ss}}) - k_{-}\langle n\rangle_{\mathrm{ss}} &= 0 \\
\langle n\rangle_{\mathrm{ss}} &= \frac{N}{1+\frac{k_{-}}{k_{+}}}
\end{aligned}$$

and the time dependent solution is easily found by separation of variables followed integration with appropriate initial condition, resulting in

$$\langle n\rangle(t) = \langle n\rangle_{\mathrm{ss}} + (\langle n\rangle_0 - \langle n\rangle_{\mathrm{ss}})e^{-(k_{+}+k_{-})t}, \qquad [2]$$

where $\langle n\rangle_0$ is the average number of units at initial time ($t = 0$). Thus, the time constant τ for the exponential approach to steady state is give by $\tau = \frac{1}{k_{-}+k_{+}}$.

B. Probability of success of stator unit assembly post-contact. As mentioned in the main text, the probability of success p_{s} depends on the contact time t_{c} between a stator unit and a rotor subunit, and the post-contact assembly rate κ. To get p_{s}, we evaluate the probability that a stator unit contacting the rotor at time $t = 0$ will successfully assemble at any time between 0 and t_{c}. The probability that the stator unit will assemble during any small interval of duration Δt is $\kappa\Delta t$ so the probability that it will not assemble during the same interval is $1-\kappa\Delta t$. The probability that the stator unit will not have assembled by the time $t_{\mathrm{c}} = N\Delta t$ is $(1-\kappa\Delta t)^N = (1-\frac{\kappa t_{\mathrm{c}}}{N})^N$. Therefore, the probability that the stator unit will have successfully assembled by time t_{c} is

$$p_{\mathrm{s}} = \lim_{N\to\infty} 1 - \left(1-\frac{\kappa t_{\mathrm{c}}}{N}\right)^N = 1 - e^{-\kappa t_{\mathrm{c}}}. \qquad [3]$$

C. Probability distribution of stator binding to the motor. The binding of a single stator unit to the motor changes its free energy by an amount $\epsilon_\mathrm{b} - \mu$, where ϵ_b is the free energy of the bound unit at zero-torque and μ is the chemical potential for taking a single free stator unit out of the membrane pool. We hypothesize that the production of torque lowers this free energy difference by an amount ϵ_T which depends on torque. Thus, the energy level of a state with n bound units is $n(\epsilon_\mathrm{b} - \mu - \epsilon_\mathrm{T})$, assuming no interaction between the bound units. The number of ways of picking n binding sites from a total of N available sites is given by the binomial coefficient $\binom{N}{n} = \frac{N!}{n!(N-n)!}$ with $n!$ denoting factorial of n. Thus, the probability of having n units bound to the motor is

$$P_n = \frac{\binom{N}{n} e^{-\beta n(\epsilon_\mathrm{b} - \mu - \epsilon_\mathrm{T})}}{\sum_{n=0}^{N} \binom{N}{n} e^{-\beta n(\epsilon_\mathrm{b} - \mu - \epsilon_\mathrm{T})}} \qquad [4]$$

where $\beta = \frac{1}{k_\mathrm{B} T}$ with the Boltzmann constant k_B and the absolute temperature T. Since that the denominator in Eq. 4 is a binomial expansion, we get

$$P_n = \frac{\binom{N}{n} e^{-\beta n(\epsilon_\mathrm{b} - \mu - \epsilon_\mathrm{T})}}{\left(1 + e^{-\beta(\epsilon_\mathrm{b} - \mu - \epsilon_\mathrm{T})}\right)^N}. \qquad [5]$$

The average steady state stoichiometry can be found by summing nP_n over all possible values of N. This leads to the average steady state occupancy

$$r = \frac{\langle n \rangle_\mathrm{ss}}{N} = \frac{1}{N} \sum_{n=0}^{N} n P_n = \frac{1}{1 + e^{\beta(\epsilon_\mathrm{b} - \mu - \epsilon_\mathrm{T})}}. \qquad [6]$$

D. Alternative models for the assembly kinetics. The observed steady state stator stoichiometry is well described by the statistical mechanical description, leading to a good agreement between measured ϵ_T and a model in which it is a linear function of the torque per stator unit Γ. Proceeding to the kinetics of stator assembly, Eq. 7 of the main text thus describes the constraint on the rate constants k_+ and k_- at the broadest level,

$$\frac{k_+}{k_-} = e^{-\beta(\epsilon_\mathrm{b} - \mu - \epsilon_\mathrm{T})}. \qquad [7]$$

We tested four different models for the dependence of the rate constant on motor's operational parameters:

1. Fixed off-rate: In this model, the off-rate k_- remains constant while the on-rate k_+ is a function of Γ, so that $k_- = k_1$ and $k_+ = k_1 e^{-\beta(\epsilon_\mathrm{b} - \mu - \lambda\Gamma)}$. For the time constant, we get $\tau = \frac{1}{k_1\left(1 + e^{-\beta(\epsilon_\mathrm{b} - \mu - \lambda\Gamma)}\right)}$. As shown in Fig. S1*A*, this model predicts τ to be a monotonically decreasing function of Γ, which does not compare well with the observations from the experiments.

2. Fixed on-rate: In this model, k_+ remains constant while k_- is a function of Γ, such that $k_+ = k_2$ and $k_- = k_2 e^{\beta(\epsilon_\mathrm{b} - \mu - \lambda\Gamma)}$. Including all data points in the fit results in a poor fit (Fig. S1*B*). However, this model does well in fitting the time scales measured from stator dissociation during the electrorotation period (Fig. S1*D*) when we excluded from the fit the data points obtained from the kinetics of re-assembly after electrorotation was turned off. This suggests that a torque-dependent off-rate is likely to be a part of the underlying kinetics but misses some key element that is relevant for the re-assembly kinetics.

3. Both on- and off-rate torque dependent: We relaxed the constraint that one of the rate constants is fixed and developed a model in which both could depend on the torque per unit Γ. The rate constants are still constrained by Eq. 7 of the main text, so we assumed that $k_+ = k_3 e^{-\beta(1-\chi)(\epsilon_\mathrm{b} - \mu - \lambda\Gamma)}$ and $k_- = k_3 e^{\beta\chi(\epsilon_\mathrm{b} - \mu - \lambda\Gamma)}$. Consequently, this model has two free parameters k_3 and χ. The best fit of this model on the time constant data still failed to capture the experimental observations (Fig. S1*C*). We further explored this model by systematically varying χ between 0 and 1, and determining a best-fit on the data with respect to k_3 (Fig. S2). While this model does result in a non-monotonic τ as a function of Γ, none of the parameter values are able to capture the experimental observations.

4. Speed dependent on-rate: As described in the main text, in this model we assumed that the on-rate decreases with increasing motor speed as $k_+ = k_0(1 - e^{-\frac{\kappa}{F}})$ where k_0 is the on-rate at stall, κ is a constant proportional to the post-contact assembly rate, and F is the motor speed in Hz. From Eq. 7 of the main text we get $k_- = k_0 e^{\beta(\epsilon_\mathrm{b} - \mu - \epsilon_\mathrm{T})}(1 - e^{-\frac{\kappa}{F}})$. The time constant τ for this model is given by $\tau = \frac{1}{k_0(1 - e^{-\frac{\kappa}{F}})(1 + e^{\beta(\epsilon_\mathrm{b} - \mu - \epsilon_\mathrm{T})})}$. A best fit captured both the dissociation and the recovery data (see main text). According to this model, τ increases rapidly with Γ for small values of Γ, and above Γ = 100 pN nm, it drops sharply. The sharp drop is caused by a rapid drop in the speed-dependent terms of τ (Fig. S3), which closely follows the trend in motor speed as a function of torque. The peak in τ occurs when the motor crosses the knee-torque and is due to the sharp bend in the torque-speed relationship. The kink in the τ vs Γ curve an artifact due to the piece-wise linear approximation for the torque-speed curve.

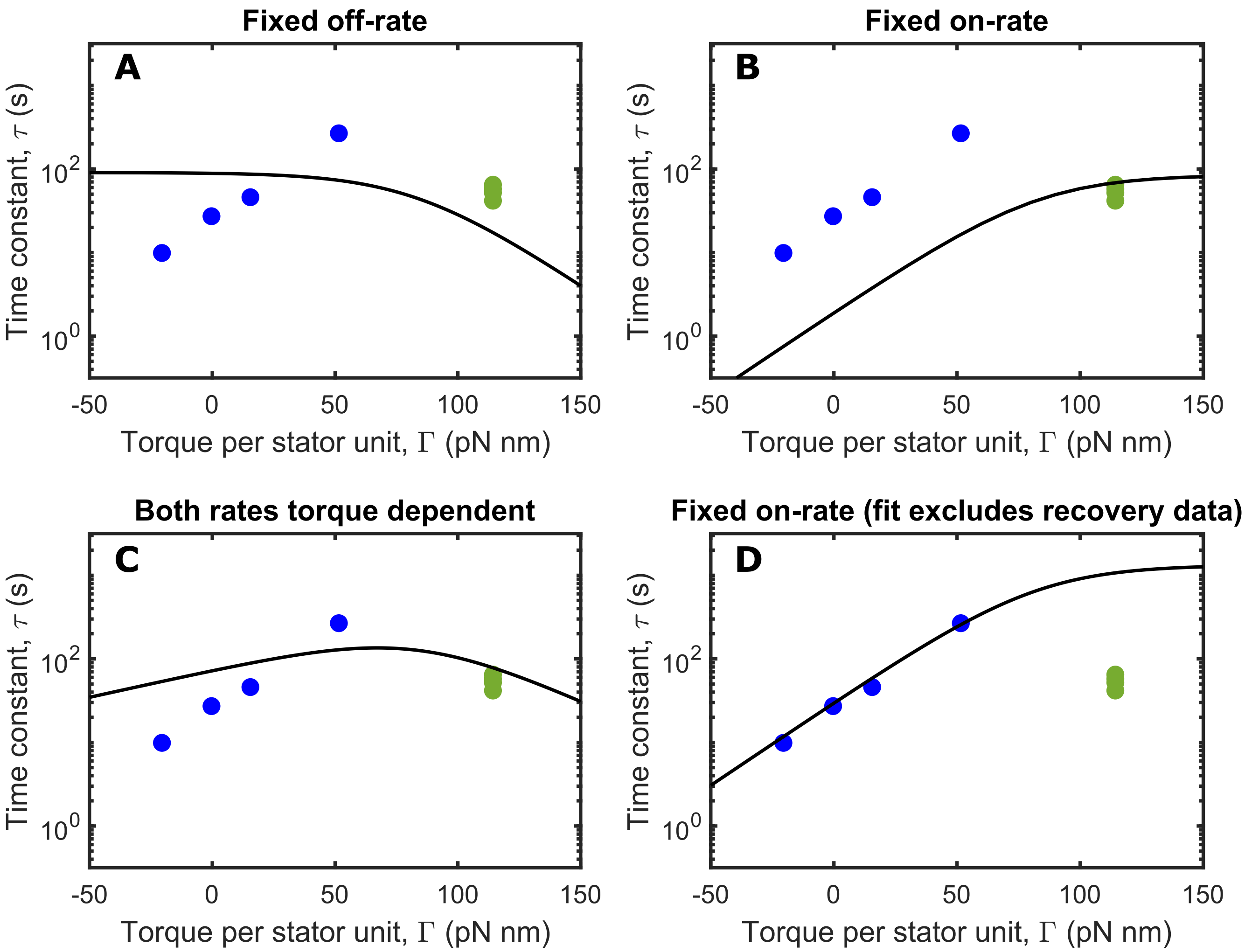

Fig. S1. Comparison between experiments and alternative models for on- and off-rates. The blue points represent data measured from dissociation kinetics and the green points are data measured from the recovery kinetics. Each curve is a best fit of the model to the data with the respective free parameters. A. The fixed off-rate model (k_1 = 0.01 s^{-1}) is the worst performer in which the time constant τ is a monotonically decreasing function of the torque per stator unit Γ. B. The fixed on-rate model (k_2 = 0.01 s^{-1}). C. The model in which both k_+ and k_- are torque-dependent (k_3 = 0.004 s^{-1} and χ = 0.33). D. The fixed on-rate model this time fit excluding the recovery data (k_2 = 0.0008 s^{-1}), to show that the fixed on-rate model is able to capture the dissociation kinetics but not the recovery kinetics.

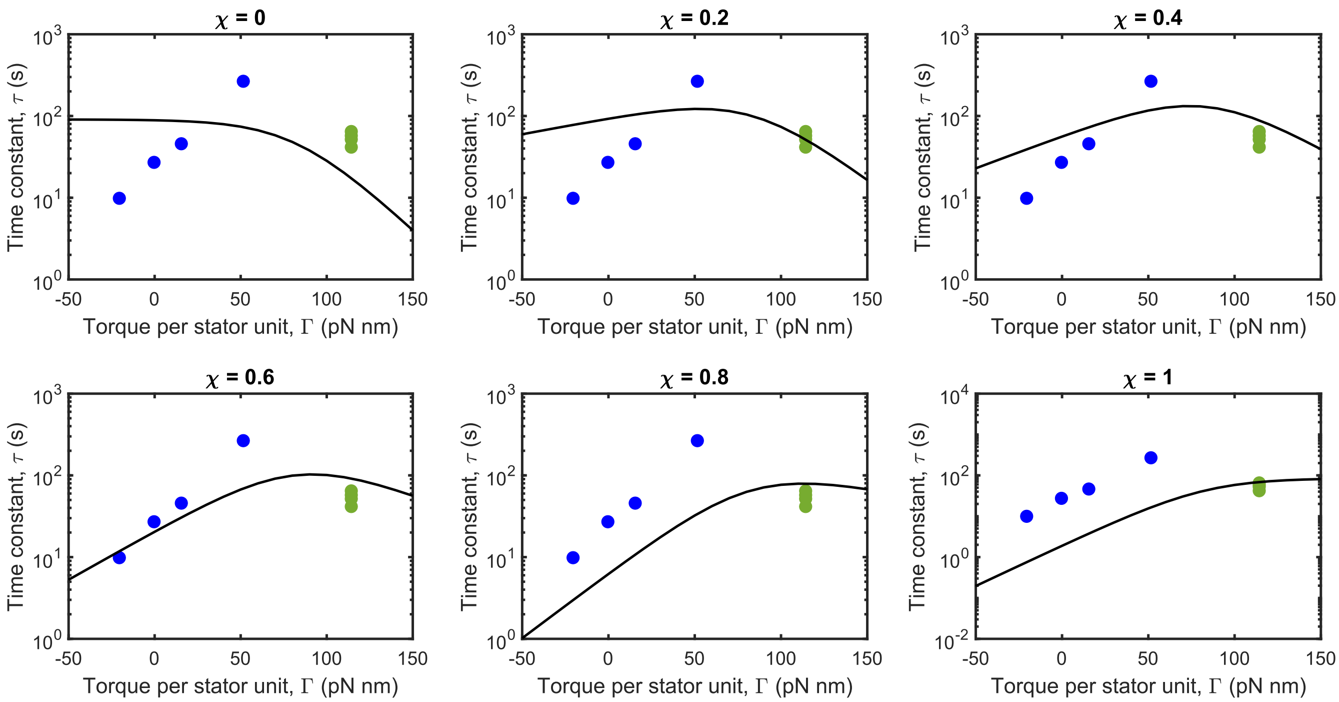

Fig. S2. Fits of the model with torque-dependent on- *and* off-rates for various values of the parameter χ. The blue points represent data measured from dissociation kinetics and the green points are data measured from the recovery kinetics. For $0 < \chi < 1$, this model results in a non-monotonic τ as a function of Γ. However, none of the parameter values in this model are able to capture all the experimental observations.

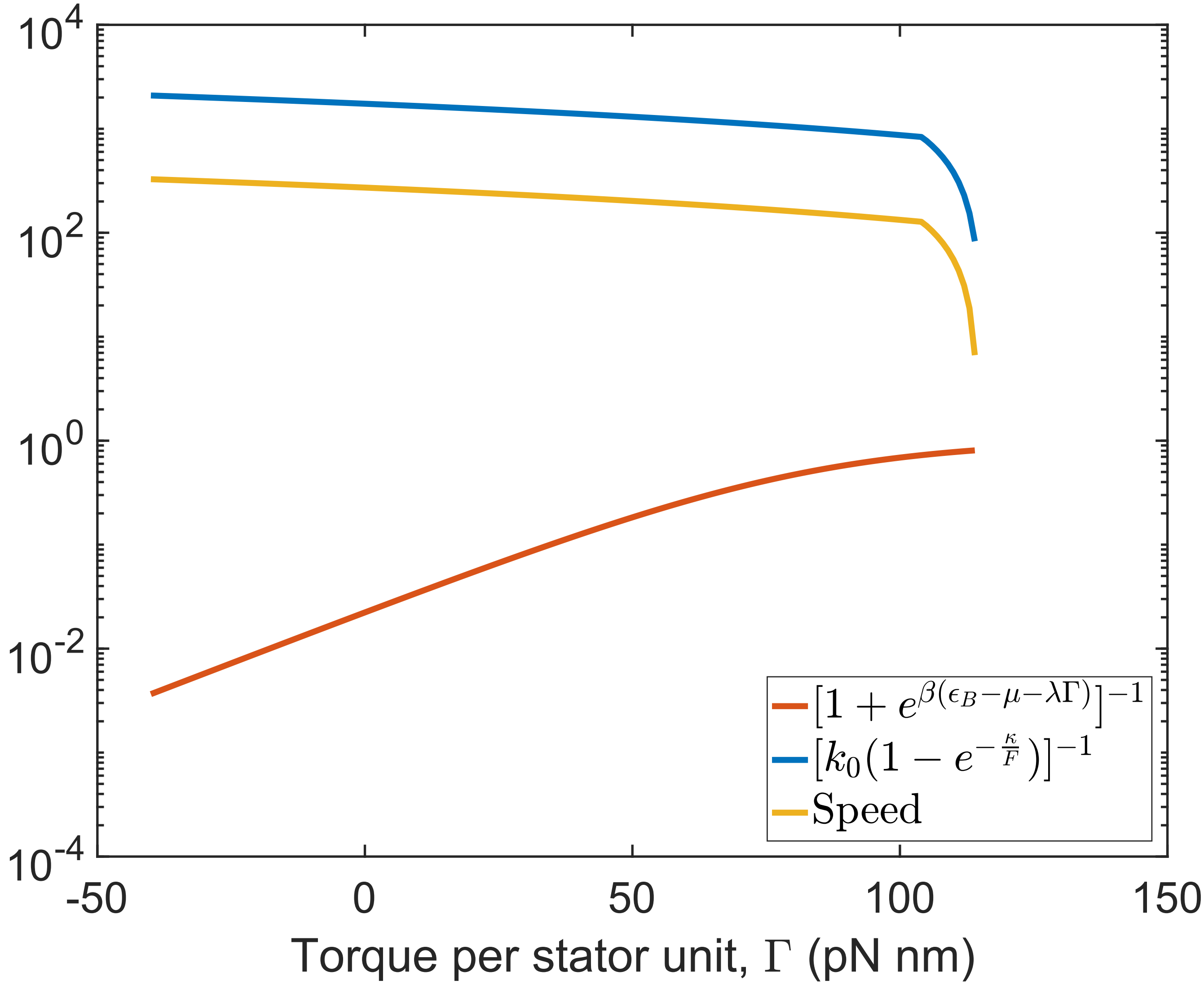

Fig. S3. Role of individual terms of Eq. 8 of the main text in the torque dependence of τ. The term $(1 + e^{\beta(\epsilon_{\mathrm{b}} - \mu - \epsilon_{\mathrm{T}})})^{-1}$, shown in red, is the primary torque dependent term which steadily increases with the torque per stator unit Γ. The other component of τ, $[k_0(1 - e^{-\frac{\kappa}{F}})]^{-1}$, shown in blue, decreases slowly until about Γ = 100 pN nm, after which it drops rapidly. The rapid drop is mainly caused by a sharp drop in motor speed above the knee-torque of about 100 pN nm, as shown in yellow.

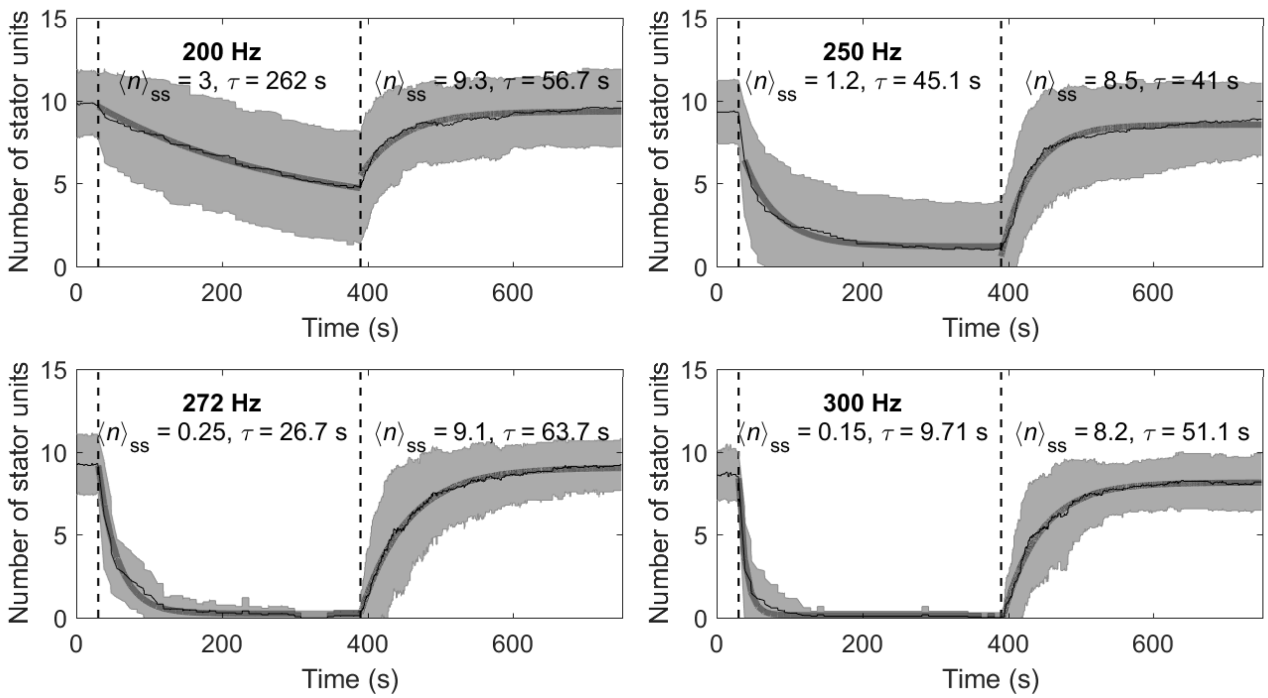

Fig. S4. Fits on the time-dependent stoichiometry data with the solution of Eq. 2 of the main text. The solid line and the shaded region are the ensemble average and the standard deviation, respectively. The grey line is the model fit with two parameters, $\langle n \rangle_{\mathrm{ss}}$ and τ, which are listed in the plots for each fit.