Fluctuations in protein synthesis from a single RNA template: Stochastic kinetics of ribosomes

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Proteins are polymerized by cyclic machines called ribosomes, which use their messenger RNA (mRNA) track also as the corresponding template, and the process is called translation. We explore, in depth and detail, the stochastic nature of the translation. We compute various distributions associated with the translation process; one of them—namely, the dwell time distribution—has been measured in recent single-ribosome experiments. The form of the distribution, which fits best with our simulation data, is consistent with that extracted from the experimental data. For our computations, we use a model that captures both the mechanochemistry of each individual ribosome and their steric interactions. We also demonstrate the effects of the sequence inhomogeneities of real genes on the fluctuations and noise in translation. Finally, inspired by recent advances in the experimental techniques of manipulating single ribosomes, we make theoretical predictions on the force-velocity relation for individual ribosomes. In principle, all our predictions can be tested by carrying out *in vitro* experiments.

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I. INTRODUCTION

A genetic message, chemically encoded in the DNA, is first *transcribed* into a messenger RNA (mRNA) from which it is then *translated* into proteins [1]. Both mRNA and proteins are linear polymers of monomeric subunits called nucleotide and amino acid, respectively. The genetic code contained in the sequence of codons (triplets of nucleotides) on an mRNA is translated into the corresponding sequence of amino acids by a macromolecular machine, called ribosome [2–4]. A ribosome is a cyclic machine. Each mechanochemical cycle of this machine consists of several steps which result in the translocation of the ribosome by one codon on the mRNA template and the elongation of the protein by one amino acid. Thus, the mRNA template also serves as the track for motorlike movement of the ribosome during translation [5,6]. In fact, a ribosome is like a mobile "workshop" which moves on an mRNA track and provides a platform where a coordinated action of many devices take place for the synthesis of each of the proteins.

Only a few papers over the last few years have reported results of single-ribosome imaging and manipulation [7–12]. These experiments have established that in each mechanochemical cycle, the dwell time of a ribosome at any codon is random. Moreover, this dwell time is a sum of two time intervals: namely, (i) the duration for which it makes a mechanical pause and (ii) the time it takes to translocate to the next codon.

In this paper we report our results on the dwell time distribution [12], which characterizes the stochastic translocation-and-pause dynamics of the ribosomes in a theoretical model of translation. We also introduce a few statistical distributions which characterize some other aspects of the stochastic nature of translation. We compute all these statistical distributions by carrying out computer simulations of a model of protein synthesis that captures both the mechanochemistry of each individual ribosome, as it moves on the mRNA template, as well as their *in situ* steric interactions [13]. This model provides a "unified" description of the stochastic dynamics of ribosomes within a single theoretical framework. In the low-density limit, it accounts essentially for the translocation-and-pause dynamics of single ribosomes because the ribosomes on the mRNA track are well separated and operate practically independent of each other. On the other hand, it also predicts the effects of steric interactions of the ribosomes on the translocation-and-pause dynamics and the emergent collective properties of the ribosomes at higher densities.

This paper is organized as follows: We present our model in Sec. II and explain how it captures the essential features of ribosome traffic on a single mRNA track. In the same section, we also describe the numerical scheme we have used for computer simulations of this model. We report the results obtained from these simulation in Sec. III. We compare some of our results with the corresponding experimental observations reported earlier in the literature and discuss ways of analyzing the data. We summarize the main results and draw conclusions in Sec. IV.

II. MODEL

Almost all the earlier works on the collective operation of ribosomes on a single mRNA track [14–24] treat ribosome traffic as a problem of nonequilibrium statistical mechanics of a system of interacting "self-driven" hard rods. But strictly speaking, a ribosome is neither a particle nor a hard rod; its mechanical movement along the mRNA track is coupled to its internal mechanochemical processes, which drive the synthesis of the protein. Thus, these earlier models could not account for the effects of the intraribosome chemical and conformational transitions on their collective spa-

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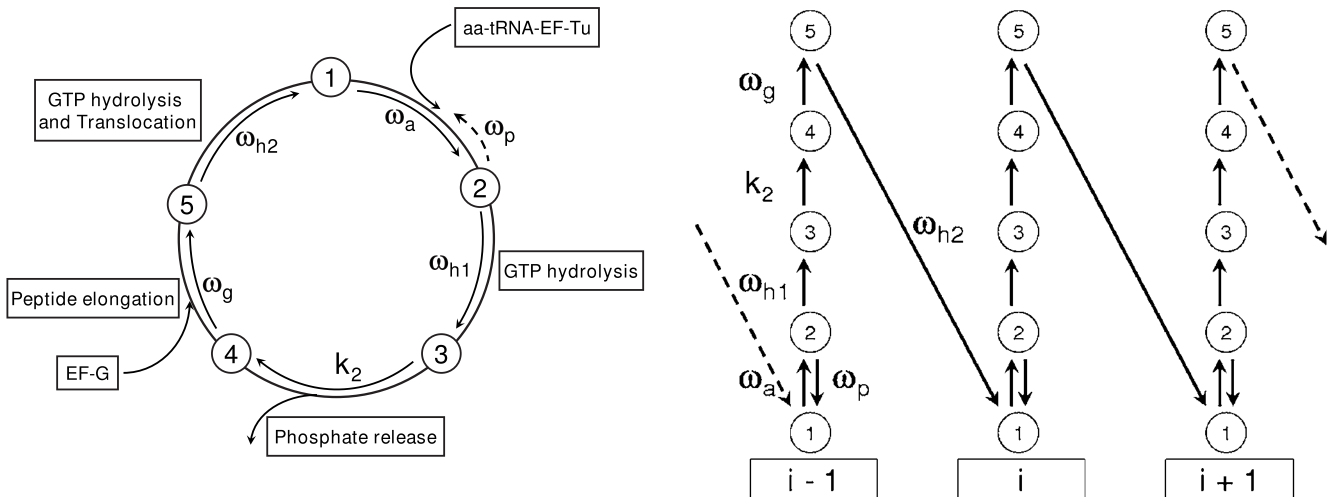

FIG. 1. A schematic representation of the biochemical cycle of a single ribosome during the elongation stage of translation in our model [13]. Each circle labeled by an integer index represents a distinct state in the mechanochemical state of a ribosome. The index below the box labels the codon on the mRNA with which the ribosome binds. The symbols accompanied by the arrows define the rate constants for the corresponding transitions from one state to another.

tiotemporal organization. In contrast, in a recently developed model [13] each of the hard rods representing individual ribosomes has several "internal" states which capture the different chemical and conformational states of an individual ribosome during its biochemical cycle.

To our knowledge, none of the earlier models of "ribosome traffic" [14–24] have been used so far to investigate any of the statistical distributions which we compute in this paper to characterize the stochastic kinetics of ribosomes. We treat the widths of these distributions as quantitative measures of "translational noise" arising from a single mRNA template. Moreover, in this paper, we demonstrate the effects of the heterogeneity of the codon sequence of real genes on this "translational noise."

We represent the single-stranded mRNA chain by a one-dimensional lattice where each site corresponds a single codon (triplet of nucleotides). The sites $i=1$ and $i=L$ represent the start codon and stop codon, respectively. Each ribosome covers ℓ sites (i.e., ℓ codons) at a time; no lattice site is allowed to be covered simultaneously by more than one overlapping ribosome because of their steric exclusion. Irrespective of the length ℓ, each ribosome moves forward by only one site in each step as it must translate successive codons one by one. We denote the position of a ribosome by the integer index of the leftmost lattice site it covers.

Figure 1(a) captures the mechanochemical cycle of each ribosome in the stage of elongation of the protein. The arrival of the correct amino acid (bound to an adapter molecule called tRNA and another protein called EF-Tu) and its recognition by the ribosome located at the site i triggers transition from the chemical state 1 to 2 in the same location with a transition rate ω_a. If codon anticodon does not match, then the tRNA can detach and makes the transition from state 2 to state 1 with transition rate ω_p. The transition from state 2 to state 3 with transition rate ω_{h1} is driven by hydrolysis of GTP. Departure of the phosphate group, which is one of the products of GTP hydrolysis, results in the intermediate state 4 with transition rate k_2. The peptide bond formation between the growing protein and the newly arrived amino acid monomer, which leads to the elongation of the protein by one amino acid monomer (and some associated biochemical processes, including the arrival of the protein EF-G), is captured by the next transition to the state 5 with transition rate ω_5. All the subsequent processes, including hydrolysis of another GTP molecule, the forward translocation of the ribosome by one codon, and the departure of a naked tRNA from the ribosome complex, are captured by a single effective transition from state 5 at site i to the state 1 at the site $i+1$ with transition rate ω_{h2}. More detailed explanations of the states and the transitions are given in Ref. [13]. The entire set of transitions and the corresponding rate constants are shown separately on the simplified Fig. 1(b) where the integer index i labels the codons on the mRNA track.

The average number of ribosomes crossing the stop codon, per unit time, on the template mRNA is called the *flux* of ribosomes. The average rate of elongation of a protein is proportional to the average velocity of a ribosome and, therefore, the flux is a measure of the total rate of synthesis of the protein encoded by the mRNA on which the ribosomes move. The flux and the average density profiles of the ribosomes on the mRNA track in our model have been reported in Ref. [13].

The time interval t_d between the arrival of a ribosome at a specific codon and its subsequent departure from there is defined as the dwell time at that codon. The run time T of a ribosome is the time it takes to run from the start codon to the stop codon on the mRNA. In other words, T is the time taken by a ribosome to synthesize a single protein. Similarly, following the terminology of traffic science [25], we identify the time interval between the departure of the successive ribosomes from the stop codon as the time headway τ. Equivalently, τ is the time interval in between the completion of the synthesis of successive proteins from the same mRNA template.

In this paper we compute the distributions $P(t_d)$, $\widetilde{P}(T)$, and $\mathcal{P}(\tau)$ of the probabilities of t_d, T, and τ. We treat the

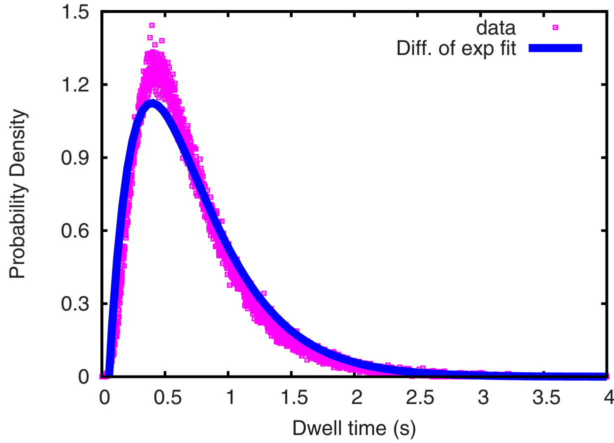

FIG. 2. (Color online) Probability distribution of the dwell times of the ribosomes for a hypothetical homogeneous mRNA template for $\ell=12$. The set of data fits well with the difference of two exponentials. The parameters are $\omega_a=\omega_g=25\ \mathrm{s}^{-1}$ and $\alpha=0.0001$.

fluctuations—i.e., root-mean-square (rms) deviations—of t_d, T, and τ as quantitative measures of noise in the translation of a single mRNA. Analogous measures of transcriptional noise have been introduced recently to characterize the stochasticity of polymerization of RNA molecules from a DNA template [26].

Simulation scheme

The results reported in this paper were obtained by computer simulations of our model. All the results of simulations reported in this paper have been obtained for $\ell=12$, which is, indeed, the typical size of a real ribosome [27]. Moreover, we imposed *open*-boundary conditions, which, for protein synthesis, are more realistic than the periodic boundary conditions. The symbols α and β denote the probabilities of attachment and detachment, respectively, in time Δt. So the probability of attachment per unit time (which we call ω_α) is the solution of the equation $\alpha=1-e^{-\omega_\alpha \Delta t}$ (in all our numerical computations we take $\Delta t=0.001$ s). Similarly, we define the corresponding parameter ω_β for termination. For the same reasons as elaborated in Ref. [13], we assume that $\omega_{h1}\simeq\omega_{h2}=\omega_h$. Moreover, throughout this article we use $\omega_h=10\ \mathrm{s}^{-1}$, $\omega_p=0.0028\ \mathrm{s}^{-1}$, $k_2=2.4\ \mathrm{s}^{-1}$, and $\beta=1$, which were used in Ref. [13] for the bacteria *E-coli*; the values of the other parameters will be given in the appropriate figure captions.

The nucleotide sequence in a real mRNA is, in general, inhomogeneous. Not all the tRNA species, which correspond to different codon species, are equally abundant in a cell; the concentrations of tRNA species which correspond to rare codons are also proportionately low [28]. We incorporate the effects of the sequence inhomogeneity in the crr gene of *Es-cherichia coli* K-12 strain MG1655 [29] in our model exactly the same way as it was done in Ref. [13]. More precisely, for a ribosome located at the ith codon, we multiply the numerical value of ω_a, which we used earlier for the hypothetical homogeneous mRNA, by a multiplicative factor that is proportional to the relative concentration of the tRNA associated with the ith codon [28,30].

We randomly select the sites on the lattice and update the state according to the dynamic rules of updating prescribed for the model. One single time step of the Monte Carlo (MC) simulation consists of updating L number of sites. We relate a single MC step with the real time by identifying the elementary time step with $\Delta t=0.001$ s. In each run of the computer simulations the data for the first 50×10^6 time steps were discarded to ensure that the system, indeed, reached steady state. The data for the computation of the dwell time distribution, run time distribution and time headway distribution were collected in the steady state over the next 50×10^6 time steps. Thus, each simulation run extended over a total of 100×10^6 time steps.

III. RESULTS AND DISCUSSION

Because of the intrinsic stochasticity of the steps of the mechanochemical cycle of the ribosome, the dwell time fluctuates even if all the codons on the mRNA track are identical. A typical distribution of the dwell times of the ribosomes during the translation of a hypothetical homogeneous mRNA is shown in Fig. 2. The numerical data obtained from computer simulations of our model can be fitted to the difference of two exponentials—i.e.,

$$P(t_d)=Ae^{-\lambda_1 t_d}-Be^{-\lambda_2 t_d}, \tag{1}$$

where the choice of the parameters $A\simeq B\simeq25$, $\lambda_1\simeq2.73\ \mathrm{s}^{-1}$, and $\lambda_2\simeq3.14\ \mathrm{s}^{-1}$ gives the best fit. The form (1) of the distribution of the dwell times is consistent with the corresponding recent experimental observation [12]. However, it is well known, that under many circumstances (for example, as shown in the Appendix), a distribution described the difference of exponentials (1) is equally well approximated by a Γ distribution

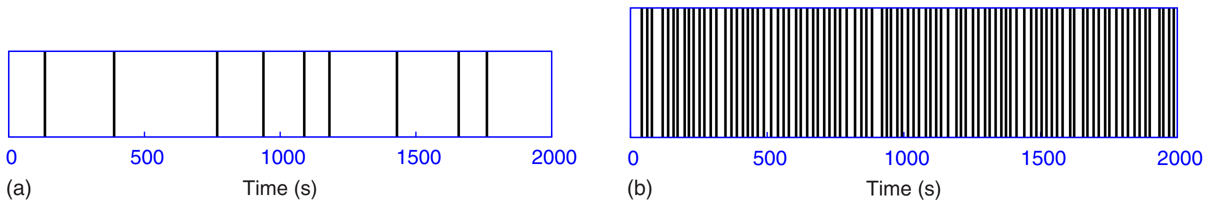

FIG. 3. (Color online) Typical time series of the translation events for (a) crr gene of *Escherichia coli* K-12 strain MG1655 and (b) the corresponding hypothetical homogeneous mRNA template, both corresponding to $\omega_a=2.5\ \mathrm{s}^{-1}$, $\omega_g=2.5\ \mathrm{s}^{-1}$, $\omega_h=10\ \mathrm{s}^{-1}$, and $\alpha=0.1$.

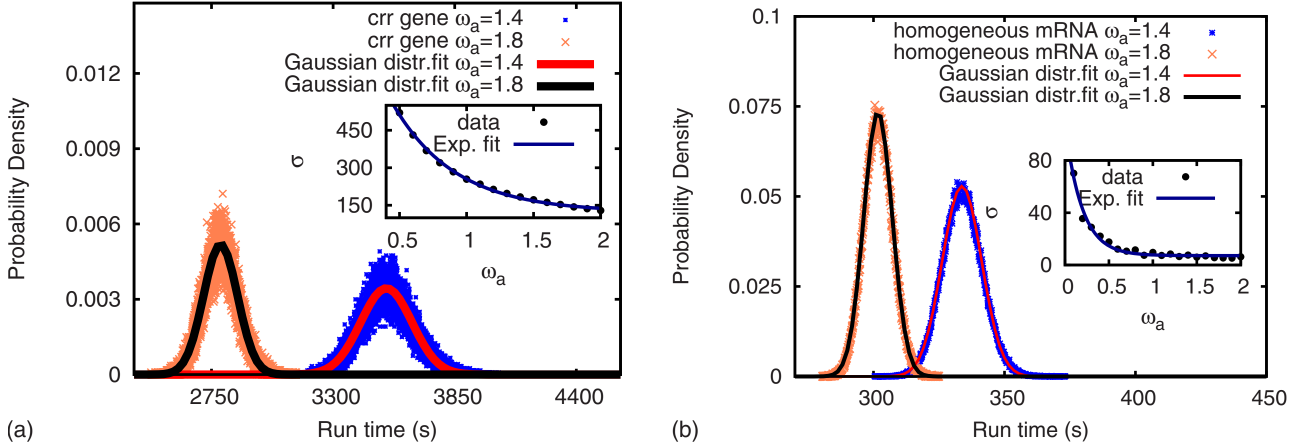

FIG. 4. (Color online) Probability distribution of the times taken to complete the synthesis of a single polypeptide (which is identical to the probability distribution of the run times of ribosomes) for (a) crr gene of *Escherichia coli* K-12 strain MG1655 and (b) the corresponding hypothetical homogeneous mRNA template. In both (a) and (b), different curves correspond to different values of ω_a, all for $\ell=12$. The discrete data points have been obtained from our computer simulations of the model whereas the lines denote the *Gaussian* best fits to these data. The insets show the exponential decrease of the corresponding noise strengths with ω_a. In both (a) and (b), $\omega_g=2.5\ \mathrm{s}^{-1}$ and $\alpha=0.1$.

$$P(t_d)=\frac{\lambda^k t_d^{k-1}e^{-\lambda t_d}}{\Gamma(k)}; \tag{2}$$

Indeed, our simulation data for the dwell time distribution fit almost equally well with the Γ distribution (2) where $\lambda \simeq 4.55\ \mathrm{s}^{-1}$ and $k\simeq 3$ (not shown in the figure).

The departure of a ribosome from the site $i=L$ (i.e., stop codon) signals the completion of synthesis of a protein; we shall call this event a *translation event*. A typical time series of the translation events is shown in Fig. 3 for the crr gene of *Escherichia coli* K-12 strain MG1655 together with a time series for the corresponding homogeneous mRNA template where all the rate constants other than ω_a are same. The longer gaps between the events for the real gene arise from the fact that a ribosome has to wait for long periods at the "hungry codons" [13]. The increase in the average time gap between the successive translation events is not surprising because it is a consequence of multiplication of the rate constant ω_a by fractions which take into account the relative abundance of the tRNA species. But what is nontrivial is the corresponding increase in the fluctuations, as we shall show in this paper.

We have plotted the distribution $\widetilde{P}(T)$ for the crr gene of the *Escherichia coli* K-12 strain MG1655, for different values of the model parameters ω_a in Fig. 4; the data for the corresponding hypothetical homogeneous mRNA template are plotted in Fig. 4(b). In Fig. 5 we have plotted the corresponding data for $\mathcal{P}_\tau$. The variation of the strength of the noise with the model parameters is shown in the insets of the respective figures. Both the measures of translational noise

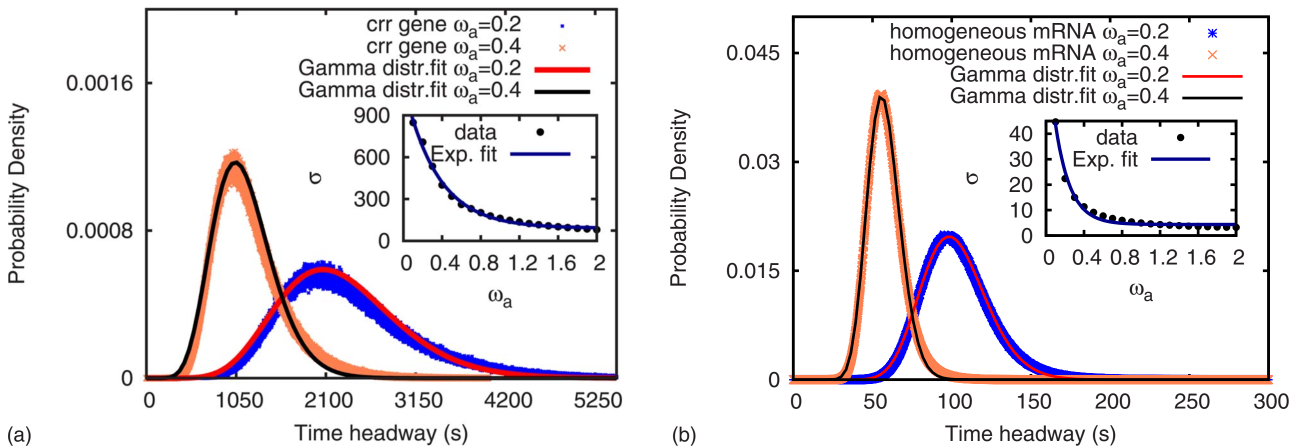

FIG. 5. (Color online) Probability distribution of the time gaps between the completions of the synthesis of a successive polypeptides (which is identical to the probability distribution of the time headways in the ribosome traffic) for (a) crr gene of *Escherichia coli* K-12 strain MG1655 and (b) the corresponding hypothetical homogeneous mRNA template. In both (a) and (b) different curves correspond to different values of ω_a, all for $\ell=12$. The discrete data points have been obtained from our computer simulations of the model, whereas the lines denote the Γ distributions fitted to these data. The insets show the exponential decrease of the corresponding noise strengths with ω_a. In both (a) and (b), $\omega_g=2.5\ \mathrm{s}^{-1}$ and $\alpha=0.1$.

fall exponentially with an increase of ω_a. In other words, an increase in the availability of the monomeric subunits (which is indicated by ω_a) reduces the noise level. A similar trend of the variation of noise with ω_h (i.e., the rate of "fuel" consumption) has been observed (but not shown graphically).

Comparing the data in Figs. 4(a) and 4(b), we conclude that the sequence inhomogeneity of real genes not only slows down the polymerization of the proteins, but also makes the process more noisy as compared to the translation of the hypothetical homogeneous gene. Similarly, comparing the data in Fig. 5(a) with those in Fig. 5(b), we establish that sequence inhomogeneity of real genes leads to a longer mean, as well as stronger fluctuations, in τ than for the hypothetical homogeneous template.

The data for $\widetilde{P}(T)$, obtained from computer simulations, fit well with a Gaussian distribution. In contrast, the best fit to those for $\mathcal{P}_\tau$ is a Γ distribution. Such long-tail distributions are quite common in gene expression and describe the characteristic features of various statistical properties of gene expression [31–33].

With the progress made in the recent years in manipulating single ribosomes, it may now become possible to measure the average speed of a ribosome as a function of an externally applied load force (a force that is applied parallel to the track and that opposes the natural motion of the ribosome). The minimum load force that stalls a motor is called the stall force, which is also a measure of the maximum force generated by a single motor. Measurements of the force-velocity relation in single-ribosome experiments will also provide estimates of the stall force for individual ribosomes.

In the *extremely-low-density* limit of our model, the average velocity v of an individual ribosome is practically identical to that of an isolated ribosome in a single-ribosome experiment. In this limit of our model, v is given by [13]

$$v = \frac{1}{\rho}\frac{\omega_{h2}\rho(1-\rho\ell)}{(1+\rho-\rho\ell)+\Omega_{h2}(1-\rho\ell)}, \tag{3}$$

where $\rho=N/L$ is the number density of the ribosomes and

$$\Omega_{h2} = \omega_{h2}/k_{eff}, \tag{4}$$

with

$$\frac{1}{k_{eff}} = \frac{1}{\omega_g} + \frac{1}{k_2} + \frac{1}{\omega_{h1}} + \frac{1}{\omega_a} + \frac{\omega_p}{\omega_a\omega_{h1}}. \tag{5}$$

The load force f affects only the mechanochemical transition from the chemical state 5 at i to the chemical state 1 at $i+1$. The dependence of the rate constant ω_{h2} on f is given by

$$\omega_{h2}(f) = \omega_{h2}(0)\exp[-f\delta/(k_BT)], \tag{6}$$

where $\omega_{h2}(0)$ is the magnitude of the rate constant ω_{h2} in the absence of the load force and $\delta=3\times 0.34$ nm is the typical length of each codon.

Using Eq. (6) in Eqs. (3) and (4), we obtain the dependence of the average velocity of the individual ribosomes on the load force f. This force-velocity relation is plotted in Fig. 6 for three different values of ω_a. The estimates of the stall forces obtained from this figure for different values of ω_a are quite realistic.

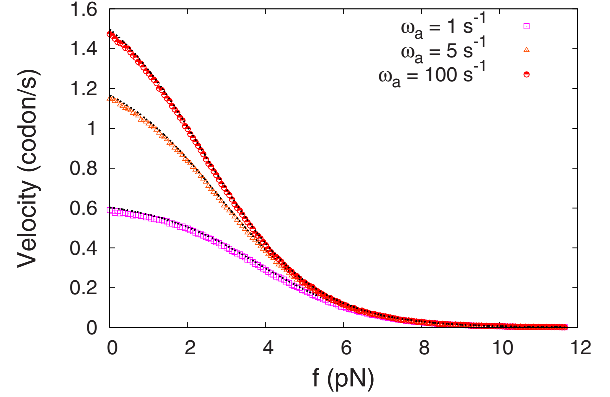

FIG. 6. (Color online) The average velocity of individual ribosomes as a function of the externally applied load force plotted for three different values of the rate constant ω_a. The solid curves have been plotted using the analytical expression (3), together with (6), for an extremely small number density of the ribosomes. The discrete data points have been obtained by computer simulations of the same model.

IV. SUMMARY AND CONCLUSION

In this paper we have developed a conceptual framework for analyzing the intrinsic stochasticity in the process of polymerization of proteins by ribosome machines from a single mRNA template. The widths of the statistical distributions, which characterize different aspects of this stochasticity, serve as quantitative measures of noise in the translation of a single mRNA. By comparing our results for a specific gene of the bacteria *Escherichia coli* with those for the corresponding artificial homogeneous mRNA template, we have demonstrated the effects of the sequence inhomogeneities of real genes on the translational noise. The nature of the dwell time distributions predicted by our theory is consistent with the corresponding observations [12] in recent single-ribosome experiments. Finally, we have also predicted the nature of the force-velocity relation for individual ribosomes. We hope our predictions will stimulate new *in vitro* single-ribosome experiments on force-velocity relation and on translational noise.

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APPENDIX: Γ DISTRIBUTION vs DIFFERENCE OF EXPONENTIALS

Let us begin with the distribution

$$P(x) = Ae^{-\lambda_1 x} - Be^{-\lambda_2 x}, \tag{A1}$$

which is a difference of two exponentials. In the special case where $A=B$ and $(\lambda_2-\lambda_1)\ll 1$, this distribution simplifies to

$$P(x) = Ae^{-\lambda_1 x}\left[(\lambda_2 - \lambda_1)x - \frac{1}{2}(\lambda_2 - \lambda_1)^2 x^2 + \cdots\right]. \tag{A2}$$

If we retain only the first-order term in $(\lambda_2 - \lambda_1)$ on the right-hand side of Eq. (A2), then the normalized distribution is given by

$$P(x) = \frac{\lambda_1^2 x e^{-\lambda_1 x}}{\Gamma(2)}, \tag{A3}$$

which is the special case $k=2$ of the standard form of the Γ distribution (2). A mixture of Γ distributions with $k=2$ and $k=3$ is obtained if both the terms linear and quadratic in $(\lambda_2 - \lambda_1)$ on the right-hand side of Eq. (A2) are retained.

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