Stretching single RNAs: exact numerical and stochastic simulation methods

Fei Liu,¹ Bi-hui Zhu,² and Zhong-can Ou-Yang¹,³

¹Center for Advanced Study, Tsinghua University, Beijing 100084, China
²Department of Physics, Peking University, Beijing 100871, China
³Institute of Theoretical Physics, The Chinese Academy of Sciences, P. O. Box 2735, Beijing 100080, China

(Dated: February 9, 2008)

Abstract

Exact numerical methods and stochastic simulation methods are developed to study the force stretching single RNA issue on the secondary structure level in equilibrium. By computing the force-extension curves on the constant force and the constant extension ensembles, we find the two independent methods agree with each other quite well. To show the precision of our methods in predicting unfolding experiments, the unfolding forces of different RNA molecules under different experimental conditions are calculated. We find that the ionic corrections on the RNA free energies alone might not account for the apparent differences between the theoretical calculations and the experimental data; an ionic correction to the persistent length of single-stranded RNA should be necessary.

PACS numbers: 82.37.Rs, 87.14.Gg, 87.15.Aa

*Email address: liufei@tsinghua.edu.cn
I. INTRODUCTION

In the past few years, enormous theoretical efforts have been devoted to understanding folding/unfolding phenomena of proteins and RNA observed in single-molecule experiments. Diverse methods including molecular dynamics [1, 2], Monte Carlo method [3, 4, 5], and other theoretical models [6] have been developed. However, these studies mainly focused on the dynamical behavior of proteins under force, and few concerned about RNA [5]. To fill this gap, we recently developed kinetic Monte Carlo simulation methods to investigate the RNA kinetic behaviors in constant force and constant extension ensembles on secondary structure level [7, 8]. In addition to the intriguing nonequilibrium phenomena, the most direct application of our simulation methods is to investigate the relative simple unfolding behaviors in equilibrium [7, 8]. Different from complex protein unfolding behaviors even in equilibrium state [9], force unfolding RNA has been showed to be solved in an exact numerical way in the constant extension ensemble [10], though the extension to the constant force ensemble were not reported till now. One of natural question arises whether our simulations are accurate enough comparing to the numerical method. In this report, we address this question.

The organization of this paper is as follows. We first, in Sec. II simply review the Monte Carlo methods developed by us. Then the exact numerical methods for the constant force and the constant extension ensembles are showed. In Sec. III we compare the simulation and numerical methods in the two ensembles. We particularly point out the importance of persistent length of RNA in predicting unfolding forces. Finally Sec. IV presents our conclusion.

II. THE MODEL AND METHODS

According to the difference of the external controlled parameters, the RNA unfolding experiments can be carried out under constant extension and constant force, i.e., the constant extension and the constant force ensembles [11]. One of apparatus for the constant extension ensemble is sketched in Fig. 1: a single RNA molecule is attached between two beads with RNA:DNA hybrid (double-stranded DNA or dsDNA) handle; one bead is held by a pipette, and the other is in a laser light trap. In practice, although two identical handles connect
the RNA, only one handle is considered in order to simplify our theoretical calculation; It should not change following discussions. By moving the position of the pipette, the distance between the two beads and the force acting on the bead in the light trap can be measured with high resolution. On the contrary, constant force can be imposed on the RNA molecules with feedback-stabilized optical tweezers capable of maintaining a preset force by moving the beads closer or further apart.

A. Monte Carlo simulation methods

The Monte Carlo algorithm is simply reviewed in this section.

First is the method for the constant extension ensemble \[^8\]. Two simplifications have been made in our model. We suppose that changes of the extensions of RNA and the handle proceed along one direction. Physical effects of the beads are neglected. Consequently, any state of the system can be specified with three independent quantities, the position of the bead with respect to the center of the optical trap, \(x^{\text{tw}}\), the end-to-end distance of the handle, \(x^{\text{ds}}\), and the RNA secondary structure \(S\), i.e. the system in \(i\)-state \((S_i, x^{\text{tw}}_i, x^{\text{ds}}_i)\). Here we do not include \(x^{\text{ss}}\), the extension of the RNA for the sum of individual extensions satisfies constraint condition, \(x = x^{\text{tw}} + x^{\text{ds}} + x^{\text{ss}}\), where \(x\) is the distance between the centers of the light trap and the bead held by the pipet, and it also is the external controlled parameter in the constant extension ensemble. The move set for this system is as follow,

\[
\begin{align*}
(S_i, x^{\text{tw}}_i, x^{\text{ds}}_i) &\rightarrow (S_j, x^{\text{tw}}_j, x^{\text{ds}}_j), i \neq j \\
(S_i, x^{\text{tw}}_i, x^{\text{ds}}_i) &\rightarrow (S_i, x^{\text{tw}}_i \pm \delta, x^{\text{ds}}_i \mp \delta), \\
(S_i, x^{\text{tw}}_i, x^{\text{ds}}_i) &\rightarrow (S_i, x^{\text{tw}}_i, x^{\text{ds}}_i \pm \delta),
\end{align*}
\]

Unfolding the single RNA for the constant extension ensemble proceeds in an extended conformational space \(C(l) \times R^{\text{tw}} \times R^{\text{ds}}\), where \(C(l)\) is the RNA secondary structural folding space, \(R^{\text{tw}} = (0, +\infty)\), \(R^{\text{ds}} = (0, l_{ds})\), and \(l_{ds}\) is the contour length of the dsDNA handle. Given the system state \(i\), its whole energy is

\[
E_i(x) = \Delta G^0_i + W^{\text{tw}}(x^{\text{tw}}_i) + W^{\text{ds}}(x^{\text{ds}}_i) + W^{\text{ss}}(x^{\text{ss}}_i, n_i),
\]

where \(\Delta G^0_i\) is the free energy obtained from folding the RNA sequence into the secondary structure \(S_i\), and the elastic energies of the optical trap, the handle, and the single-stranded
part of the RNA are

\[
W^{tw}(x_i^{tw}) = \frac{1}{2} k_{tw} x_i^{tw^2},
\]

\[
W^{ds}(x_i^{ds}) = \int_{x_i^{ds}}^{x_i^{ds}} f_{ds}(x') dx',
\]

\[
W^{ss}(x_i^{ss}, n_i) = x_i^{ss} f(x_i^{ss}, n_i) - \int_{0}^{f(x_i^{ss}, n_i)} x_{ss}(f', n_i) df',
\]

respectively. In the expression \( W^{ds} \), \( f_{ds}(x') \) is the average force of the handle at given extension \( x' \),

\[
f_{ds}(x') = \frac{k_B T}{P_{ds}} \left( \frac{1}{4(1 - x'/l_{ds})^2} - \frac{1}{4} + \frac{x'}{l_{ds}} \right),
\]

where \( P_{ds} \) is the persistence length, respectively. In the expression \( W^{ss} \), \( x_{ss}(f', n_i) \) is the average extension of the single stranded part of the RNA whose bases (exterior bases) is \( n_i \) at given force \( f' \),

\[
x_{ss}(f', n_i) = n_i b_{ss} \left[ \coth \left( \frac{f l_{ss}}{k_B T} \right) - \frac{k_B T}{f l'_{ss}} \right],
\]

where \( b_{ss} \) and \( l_{ss} \) are the monomer distance and the Kuhn length of the single-stranded RNA, respectively \([12, 13]\). Note that \( f(x_i^{ss}, n_i) \) is the inverse function of \( x_{ss}(f', n_i) \).

Then is the simulation method for the constant force ensemble \([7]\). We proposed an energy expression on the coarse-grain level for the given secondary structure \( S_i \) under constant force \( f \),

\[
E_i(f) \approx \Delta G_i^0 - n_i \times g(f),
\]

where \( g(f) = k_B T b_{ss}/l_{ss} \ln \sinh(u)/u \) and \( u = l_{ss} f / k_B T \). In contrast to the constant extension ensemble, the RNA secondary structure \( S \) can completely specify any state of the constant force ensemble. Therefore, the move set for this ensemble is the same with the set for RNA folding without force, i.e., its unfolding space is \( C(l) \).

Given the move sets and the unfolding conformational spaces, the RNA unfolding for the two ensembles can be modelled as a Markov process in their respective spaces. Define the transition probabilities \( k_{ij} \) from i-state to j-state satisfying \( k_{ij} = \tau_o^{-1} \exp(-\Delta E_{ij}/2k_B T) \), or the symmetric rule \([14]\), where \( \Delta E_{ij} = E_j - E_i \), \( \tau_o \) is used to scale time axis of the unfolding process. We use a continuous time Monte Carlo algorithm to simulate unfolding process \([16, 17]\).
The measurement quantities $\langle A \rangle$ for the two ensembles can be calculated by $\langle A \rangle(n) = \sum_i A_i(t_{i+1} - t_i)$, where $A_i$ is the $A$-value in state $i$, and $t_i$ is the inner time of the Monte Carlo simulations. For the constant extension ensemble, $A$ could be the force exerted on the bead, $f = k_{tw} x_{tw}$ in the light trap, or the bead-to-bead distance $x^{bb} = x^{ds} + x^{ss}$. While for the constant force ensemble, $A$ is the molecular extension $x$ under the constant force $f$, and $x_i = x_{ss}(f, n_i)$. The simulation time is $2 \times 10^6 \tau_0$.

B. the exact numerical methods

Compared to difficult protein folding prediction, the RNA secondary structure prediction has achieved great success [18]. In particular the partition function method developed later provided strongly physical foundation [19]. Recently, this method was generalized to the case of RNA unfolding in the constant extension ensemble [10]. In the present work, we are not ready to choose the formulae presented in Ref. [10]. In addition to be consistent with the formulae for the Monte Carlo simulations, the complicated polymer model of single-stranded DNA (ssDNA) therein might not result in many advances in predicting and understanding the RNA unfolding phenomena.

The key idea of the partition function method is that the partition function over all secondary structures of a given RNA can be calculated by dynamic programming. Given the partition function $Q(i, j, n)$ on the sequence segment $[i,j]$ with exterior bases $n$, its recursion formula is as follows,

$$Q(i, j, n) = \delta_{k,j-i+1} + q_{b(i, \Delta + j - n)}$$
$$+ \sum_{k=i}^{j-1} \sum_{m=1}^{k-i+1} Q(i, k, m)$$
$$\times q_{b(k+1, m + \Delta + j - n)}, \tag{7}$$

where the partition function $q_{b(i, j)}$ on the sequence segment $[i,j]$ for which the $i$ and $j$ bases are paired; Vienna package 1.4 provides their calculation codes [20].

For a given RNA sequence consisting of $N$ nucleotides, define the total partition function for the constant extension and the constant force ensemble $Z_N(x)$ and $Z_N(f)$, respectively.
According the energies mentioned in last section, their expressions can be written as

\[
Z_N(x) = \sum_n \int_0^{l_{ds}} dx^{ds} \int_0^{l_{ss}} dx^{ss} Q(1, N, n) \times \exp(-\beta E(x, x^{ds}, x^{ss}, n))
\]

and

\[
Z_{N}(f) = \sum_n Q(1, N, n) \exp(-\beta E(f, n))
\]

where the elastic energy

\[
E(x, x^{ds}, x^{ss}, n) = W^{tw}(x - x^{ds} - x^{ss}) + W^{ds}(x^{ds}) + W^{ss}(x^{ss}, n)
\]

and

\[
E(f, n) = n \times g(f).
\]

Correspondingly, the measurement quantities for the constant extension ensemble are the average force

\[
\langle f \rangle = -k_B T \frac{\partial Z_N(x)}{\partial x}
\]

and the average extension

\[
\langle x^{bb} \rangle = x - \langle f \rangle / k_{tw}, \quad \text{and} \quad \langle x \rangle = k_B T \frac{\partial Z_N(f)}{\partial f}
\]

for the constant extension ensemble, respectively.

III. COMPARISON OF THE EXACT AND SIMULATION METHODS

To compare the exact and simulation methods discussed above, we calculate extension-force curves of three small RNA, p5ab, p5abcΔA and p5abc in equilibrium. Their native states under experimental condition are showed in Fig. 1. These molecules have been studied by the experiment [11] and simulation [7, 8]. We first choose the widely used parameters for our computation: temperature \( T = 298 \) K, \( b_{ss} = 0.56 \) nm, \( l_{ss} = 1.5 \) nm, \( P_{ds} = 53 \) nm [12, 13], \( l_{ds} = 320 \) nm, and \( k_{tw} = 0.2 \) pN/nm [11], and the free energy parameters for RNA secondary structures at standard salt concentrations: \([Na^+] = 1M\) and \([Mg^{2+}] = 0M\) [20].

Fig. 2 shows these extension-force curves for the sequences for the two ensembles. We find that the two independent methods achieve highly consistence. In particular, the three curves of the molecules for the constant extension ensemble also agree with the experimental measurements very well in quantity: the extensional transition of P5ab are all-or-none; while P5abc has an intermediate state [11]. Interestingly, we note that, P5abcΔA although has been observed as two-state molecule in the experiment, a weaker intermediate state presents in the constant extension ensemble, while it cannot be observed in the constant force ensemble.

If we purchase the precision of our methods, quantitative comparison between the theoretical molecular unfolding force \( f_u \) and the experimental measurements of course is essential.
But we find that they do not coincide: in the constant extension ensemble, the experimental unfolding force of P5ab is 13.3 pN, of p5abcΔA is 11.4 pN, and of P5abc is 8 pN [11]; while our calculations are 18.4 pN, 15.8 pN and 12.2 pN, respectively. So what causes result to the larger differences between the experiment and the theory? The experiment and previous theoretical works contributed the differences to the change of free energy of the RNA secondary structure; this change results from the different ionic concentration of the experiment and the standard condition: in the RNA unfolding experiment, Na\(^+\) = 250 mM and with and without Mg\(^{2+}\) = 10 mM [10, 11, 21]. To reproduce the experimental ionic condition, a correction on the energy of a base pair equal to \(-0.193k_BT\ln(\left[Na^+\right] + 3.3[MG^{2+}]^{1/2})\) has been applied [21]. Their values are summarized in Tab. I. Besides the three molecules from Ref. [11], other unfolding forces of the molecules published in the lectures [22, 23] are also listed there. We still see that the ionic correction cannot explain the derivation between the theory and experiment.

Considering that the free energy parameters of the RNA secondary structure were measured in bulk experiments, one might doubt whether they can be used in single-molecule studies as well as we thought before [8]. On the other hand, however, it is known that the mechanical parameter, the persistent length \(l_{ss}\) is also sensitive to ionic condition. Although this parameter indeed were measured under a similar experimental conditions with the small RNA unfolding experiment (see Ref. [12]), their validity for describing small molecules is questionable. Recent FRET experiment measured that for shorter ssDNA \(l_{ss}\) is about 2.2 nm at Na\(^+\) = 250 mM [24]. If we choose this value in our calculation, the predicted unfolding forces are closer with the experimental measurements; see Tab. I. Of course, we cannot exclude the intrinsic limitation of our coarse-grain model. For example, another possible force work formula has been used in the constant force ensemble [7].

IV. CONCLUSION

In this work, we review the Monte Carlo methods and develop the exact numerical methods to study the force stretching single RNA molecules issue. We respectively compare the two independent method in the constant force and extension ensembles, and find that they agree with each other quite well. We also point out that only ionic correction on the RNA secondary structure alone cannot explain the larger discrepancies of the unfolding forces.
between the theoretical prediction and the experimental measurement; the ionic correction on the RNA molecular mechanical properties should be important.

Although the results of the exact numerical method are consistent with the Monte Carlo method when force stretches single RNA in equilibrium, it does not mean the former can completely replace the later. Such situation is similar with the study of 2-dimension Ising model in condense matter physics [25]. Compared to the exact method, the Monte Carlo method would be more sophisticate in dealing possible more complicated experimental condition. For instance, recent simulation work could include pseduknots structure [26], while the exact partition function technique would be hardly to realize. In our point of view, Monte Carlo simulation is more important in studying single molecular non-equilibrium behavior produced by mechanical force, such as folding/unfolding trajectories, force-hysteresis phenomena and unfolding force dependance on loading rates etc [7, 8].

We thank Professor H.-W. Peng for many helpful discussions in this work.

[1] H. Lu et al., Biophys. J. 75, 662 (1998).
[2] Z. Bryant, V. S. Pande, and D. S. Rookhsar, Biophys. J. 78, 584 (2000).
[3] D. K. Klimov and D. Thirumalai, Proc. Natl. Acad. Sci. USA 96, 6166 (1999).
[4] N. D. Socci, J. N. Onuchic, and P. G. Wolynes, Proc. Natl. Acad. Sci. USA 96, 2031 (1999).
[5] S. Harlepp et al., Eur. Phys. J. E 12, 605 (2003).
[6] G. Hummer and A. Szabo, Biophys. J. 85, 5 (2003).
[7] F. Liu and Z.-C. Ou-Yang, Phys. Rev. E 70, 040901 (2004)
[8] F. Liu and Z.-c. Ou-Yang, Biophys. J. (to be published).
[9] T. Shen, L.S. Canino, and J.A. McCammon, Phys. Rev. Lett. 89, 068103 (2002).
[10] U. Gerland, R. Bundshuh and T. Hwa, Biophys. J. 81, 1324 (2001).
[11] J. Liphardt et al., Science 292, 733 (2001).
[12] S. B. Smith, Y. Cui, and C. Bustamante, Science 271, 795 (1996).
[13] C. Bustamante, J.F. Marko, E.D. Siggia, and S.B. Smith, Science 265, 1599 (1994).
[14] K. Kawasaki, Phys. Rev. 145, 224 (1966).
[15] C. Flamm, W. Fontana, I. Hofacker, and P. Schuster, RNA 6, 325 (2000).
[16] A.B. Bortz, M.H. Kalos, and J.L. Lebowitz, J. Comp. Phys. 17, 10 (1975).
[17] D. T. Gillespie, J. Comp. Phys. 22, 403 (1976).
[18] M. Zuker, P. Stiegler, Nucl Acid Res 9, 133 (1981).
[19] J.S. McCaskill, Biopolymers 29, 1105 (1990).
[20] I.L. Hofacker et al., Monatshefte f. Chemie. 125, 167 (1994).
[21] S. Cocco, J. F. Marko, and R. Monasson, Eur. Phys. J. E 10, 153 (2003).
[22] M. Rief, H. Clausen-Schaumann and H.E. Gaub, Nature struct. Biol. 6, 346 (1999).
[23] C. Bustamante, S.B. Smith, J. Liphardt and D. Smith, Curr. Opin. Struct. Biol. 10, 279 (2000).
[24] M.C. Murphy et al., Biophys. J. 88, 2530 (2004).
[25] Newman, M.E.J. and G.T. Barkema. Monte Carlo Methods in Statistical Physics, (Clarendon Press, Oxford, 1999).
[26] Isambert, H. and E.D. Siggia, Proc. Natl. Acad. Sci. USA 97, 6515 (2000).
TABLE I: The unfolding forces $f_u$ of different molecules under different experimental conditions. The experimental data are from the previously published data \[11, 22, 23\]. The theoretical values are from the exact numerical methods developed above, where $f_u^i$, $i = 1, 2, 3$ represent the unfolding forces without the ionic correction, with the ionic correction on the free energy and with the ionic and the persistent length corrections, respectively. Here we do not show the P5abc unfolding force for it is not reversible in $Mg^{2+}$ due to the presence of tertiary interactions.

| Molecule       | temperature (K) | $Na^+$ (mM) | $Mg^{2+}$ (mM) | $f_u^1$ (pN) | $f_u^2$ (pN) | $f_u^3$ (pN) | $f_u^{exp}$ (pN) |
|----------------|----------------|-------------|---------------|--------------|--------------|--------------|-----------------|
| P5abc          | 298            | 250         | 0             | 12.2         | 11.4         | 10.0         | 7.0-11.0        |
| poly(dA-dU)    | 293            | 150         | 0             | 12.3         | 11.0         | 9.3          | 9.0             |
| P5abcΔA        | 298            | 250         | 0             | 15.8         | 14.8         | 13.2         | 11.4 ± 0.5      |
| P5abcΔA        | 298            | 250         | 10            | 15.4         | 13.8         | 12.7 ± 0.3   |                 |
| P5ab           | 298            | 250         | 0             | 18.4         | 17.4         | 15.7         | 13.3 ± 1.0      |
| P5ab           | 298            | 250         | 10            | 18.0         | 16.2         | 14.5 ± 1.0   |                 |
| CG hairpin     | 293            | 150         | 0             | 25.8         | 24.4         | 22.4         | 17.0            |
| poly(dC-dG)    | 293            | 150         | 0             | 25.1         | 23.8         | 21.7         | 20.0            |
FIG. 1: Theoretical model and RNA sequences and their native structures studied in present work. The structures are folded by Vienna RNA package 1.4. The equilibrium and kinetic behaviors of these three RNAs, p5ab, p5abcΔA, and p5abc have been studied in detail [11].
FIG. 2: Comparison of the exact and simulation force-extension curves in equilibrium for P5ab, P5abcΔA and P5abc in the two ensembles. The different symbols are from the simulation methods, and the different lines are from the exact methods. They agree with each other very well.