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Short Communication

RNA polymerase pushing

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ABSTRACT

Molecular motors can exhibit Brownian ratchet or power stroke mechanisms. These mechanistic categories are related to transition state position: An early transition state suggests that chemical energy is stored and then released during the step (stroke) while a late transition state suggests that the release of chemical energy rectifies thermally activated motion that has already occurred (ratchet). Cellular RNA polymerases are thought to be ratchets that can push each other forward to reduce pausing during elongation. Here, by constructing a two-dimensional energy landscape from the individual landscapes of active and backtracked enzymes, we identify a new pushing mechanism which is the result of a saddle trajectory that arises in the two-dimensional energy landscape of interacting enzymes. We show that this mechanism is more effective with an early transition state suggesting that interacting RNAPs might translocate via a power stroke.

1. Introduction

DNA transcription is a ubiquitous process in all biological organisms and serves as the primary link between genetic information and phenotype. Central to this process is the molecular machine RNA polymerase (RNAP) which is responsible for reading the DNA sequence and synthesizing an RNA copy. Numerous mechanistic and structural studies of this motor enzyme have revealed the complex series of molecular reorganizations that occur during a single cycle of NTP binding, NTP hydrolysis, RNA chain elongation and forward translocation along the DNA template [1–7]. A variety of pause states with different characteristic lifetimes and different translocation registers relative to the active configuration interrupt the smooth forward progress of the polymerase [8,9]. Of particular interest is the backtracked state, where the enzyme spontaneously loses track of the 3′ end of the RNA and slides backwards (upstream) along the DNA template [10–13]. This state occurs stochastically in a sequence biased manner, may be induced by physical barriers, error incorporation or the application of opposing force [10,11,14] and results in the creation of a passively diffusing state where the polymerase may no longer use the energy from NTP hydrolysis to bias its motion forwards [15].

One effect that has been suggested to contribute to transcriptional regulatory mechanisms is the interaction between multiple polymerases on the same template [16–18]. In analogy to traffic jams, one might expect that transit rates would decrease as the density of polymerases increases [19]. Contrary to that expectation, biochemical experiments have shown that active polymerases are able to rescue stalled backtracked enzymes in front of them and increase the efficiency of transcription through site-specific DNA binding proteins or paused states both in vitro and in vivo [20–23]. Furthermore, recent work has shown that ribosomes translating behind RNA polymerase in bacteria also function to reduce polymerase pausing [24]. These observations suggest that an active polymerase or ribosome uses its NTP-dependent translocation activity to push a backtracked polymerase forward and speed its recovery. Here, we look closer at this pushing hypothesis and show how different mechanisms of translocation lead to different levels of polymerase pushing.

2. Model of polymerase pushing

We describe the behavior of individual enzymes as resulting from motion on a one-dimensional free energy landscape. The motion of an active polymerase is coupled to NTP hydrolysis so that a single base-pair step results in a lower free energy. A backtracked enzyme behaves as a passive diffusive particle so that a step in either direction is isoenergetic to a first approximation. We thus have two interacting particles, a diffusing one (i.e. the backtracking polymerase) that is trailed by an actively stepping one.

The pushing mechanism can be illustrated by deriving a discrete kinetic model from the continuous free-energy landscape that results from the interaction between the motor (active polymerase) and the

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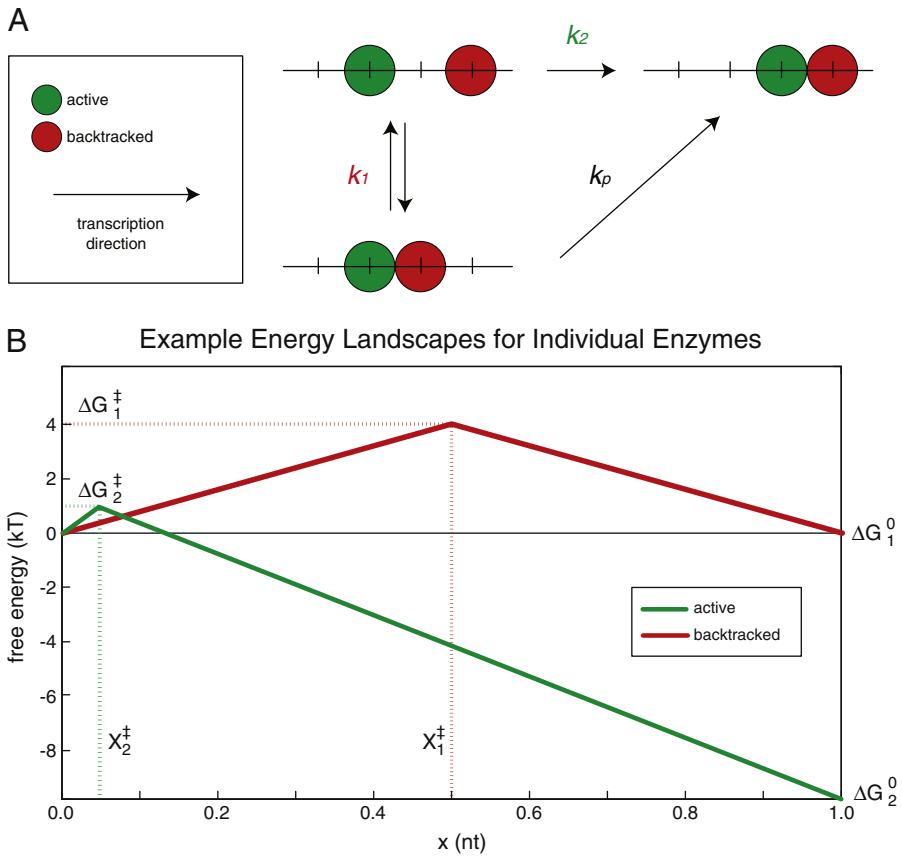

Fig. 1. Model of RNA polymerase pushing. (A) Kinetic scheme of possible moves for two enzymes that have encountered one another on the DNA template where a backtracked enzyme (red) is in front of an active enzyme (green). Two paths that lead to both enzymes stepping forward are shown. (B) An example of the piecewise linear energy landscapes for the stepping of backtracked (red) and active (green) enzymes. Each landscape consists of three parameters: the transition state free energy and position ($\Delta G^{\ddagger}$ and $x^{\ddagger}$) and the step energy (ΔG^{0}).

obstacle (backtracked polymerase). We investigate the two enzyme system shown in Fig.1. Once the enzymes encounter one another, there are two possibilities. The first is shown by the sequential path k_1k_2 where the backtracked enzyme steps forward on its own using energy from the bath (k_1) and then the active enzyme catches up (k_2) (referred to as the passive path). The second possibility is that the active enzyme steps forward and while doing so, pushes the backtracked enzyme forward at the same time (k_P) (referred to as the pushing path). The energy landscapes for each enzyme are constructed in a piecewise linear way. Each individual enzyme landscape is completely determined by three parameters (supplemental information), namely the transition state position ($x^{\ddagger}$), the transition state energy ($\Delta G^{\ddagger}$) and the final energy (ΔG^{0}) where all positions are given in base-pairs and all energies are given in units of kT. We assume that the backtracked enzyme diffuses along an isoenergetic landscape ($\Delta G_1^0 = 0$). Clearly, sequence variations will render this assumption incorrect, but these variations do not affect the conclusions of the analysis nor the essence of the implications for motor interaction and enzyme pushing.

The two enzyme system evolves on a free energy landscape that is given by the sum of the individual landscapes plus an interaction term:

$$G(x_1, x_2) = G_1(x_1) + G_2(x_2) + G_{12}(x_2 - x_1) \quad (1)$$

where x_1 and x_2 denote the positions of each enzyme (Fig. 2A–C). If we assume that enzymes act as hard spheres, $G_{12}(x_2-x_1)$ is zero if $x_1 > x_2 + d$, where d describes the extent of the enzyme, and infinity otherwise. Using a soft repulsive potential with a range that is smaller than the extent of a base-pair does not change the general conclusions that follow. For long ranged potentials other pushing mechanisms have been reported, see discussion below [25–27]. While the system is free to choose any path across the landscape, two limiting cases may be used to compare the two possibilities illustrated in Fig. 1A. Specifically, the backtracked enzyme may take a step forward on its own (vertical gray arrow, Fig. 2A–C) or the active enzyme may take a step forward and push the backtracked enzyme at the same time (diagonal blue arrow, Fig. 2A–C). We then look at the free energy differences along each of these paths (Fig. 2D–F) to estimate relative rates using transition state theory [28,29]. By varying the parameters of the isolated enzyme landscapes (Fig. 1B), we observe how different transition state positions lead to different rates along the pushing path. For example, keeping all other parameters fixed, we vary the position of the transition state for the active enzyme ($x_2^{\ddagger}$) as shown in Fig. 2.

3. Results and discussion

3.1. Enzymes in phase (integer d)

We first assume the extent of a polymerase to amount to an integer multiple of the step size of a base-pair. Three exemplary sets of free energy landscapes are shown in Fig. 2A–C. In Fig. 2D–F, the free energy along the pushing path (blue) is compared to the passive path (gray). The maximum energies along each path are highlighted with colored dots. In this set of parameters, only the landscape with an early transition state position, $x_2^{\ddagger} = 0.2$ (Fig. 2A and D), displays an energy barrier along the pushing path (blue) that is lower than that for the passive motion of the diffusive enzyme alone (gray). The two-dimensional energy landscapes illustrate that a true pushing path (i.e. a saddle trajectory along the diagonal) only exists for $x_2^{\ddagger} < x_1^{\ddagger}$, when the

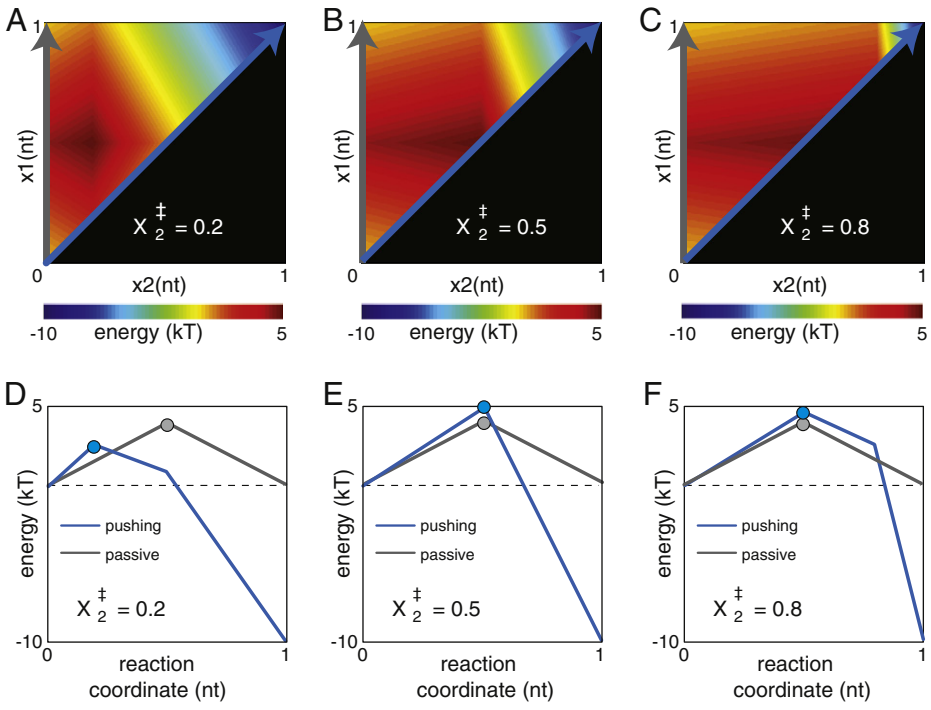

Fig. 2. Two dimensional landscapes for $x_1^{\ddagger}=0.5$, $\Delta G_1^{\ddagger}=1$, $\Delta G_1^0=0$, $\Delta G_2^{\ddagger}=4$, $\Delta G_2^0=-10$ and three different values for $x_2^{\ddagger}$. (A–C) Contour plots of energy versus active (x-axis) and backtracked (y-axis) enzyme position for different positions of the active enzyme transition state ($x_2^{\ddagger}$). The reaction coordinate span a single base pair step forward for each enzyme. Red areas of the plots are the highest in energy and represent the transition state for the coupled system while blue regions are energy minima. (D–F) One dimensional slices from the corresponding 2D plots above for different values of $x_2^{\ddagger}$. The stepping of the backtracked enzyme alone is shown in gray while the diagonal path representing the path for pushing is shown in blue. Energy maxima used for calculating rates for each path are indicated with gray or blue dots respectively.

maximum of the free energy, i.e., the point $[x_1^{\ddagger}, x_2^{\ddagger}]$ lies above the diagonal. Moreover, the point at which the transition state energy of the pushing path becomes less than that of the passive path can be calculated and provides an estimate of when the pushing path will become more probable than the passive one (supplemental information):

$$x_2^{\ddagger} < \left[1-\frac{\Delta G_2^{\ddagger}}{\Delta G_1^{\ddagger}}\right] x_1^{\ddagger} \tag{2}$$

Pushing will always occur with a probability given by the ratio of the pushing rate (k_P) to the sum of rates (k_P+k_1). We calculate this ratio using the relative heights of the transition states for each path (supplemental information). The probability of pushing and the ratio k_P/k_1 for different values of $x_2^{\ddagger}$ and ΔG_2^0 confirm what our previous arguments suggest: appreciable pushing only occurs with early transition states and large reaction energy for the active polymerase (Fig. 3). In this region and for the parameter set used in this example ($\Delta G_1^{\ddagger}=1$, $x_1^{\ddagger}=0.5$, $\Delta G_1^0=0$, $\Delta G_2^{\ddagger}=4$), the probability of pushing becomes close to one. A more realistic scheme where the pushing enzyme can also enter a backtrack makes pushing less likely, resulting in a further narrowing of the region of the parameter space that leads to pushing (supplemental information).

3.2. Enzymes out of phase (non-integer d)

We next discuss the more realistic case where the size (d) of the polymerase is not an integer number of base-pairs. In this case, when the active and trailing polymerases are positioned at a minimum of their respective free energy landscapes and as close to each other as possible, there will be a physical gap Δx in between them. We next performed the same analysis as above, but average over possible gap sizes ($0<\Delta x\leq 1$, Fig. 4A) (supplemental information). We calculate the interactive energy landscapes using the same method as before and repeat the calculations of rate ratios while varying both the gap size (Δx) and the transition state position of the active enzyme ($x_2^{\ddagger}$). This allows us to calculate the gap-averaged rate ratios as a function of transition state position (Fig. 4B). The curve reiterates our previous conclusions that early transition states lead to more efficient pushing.

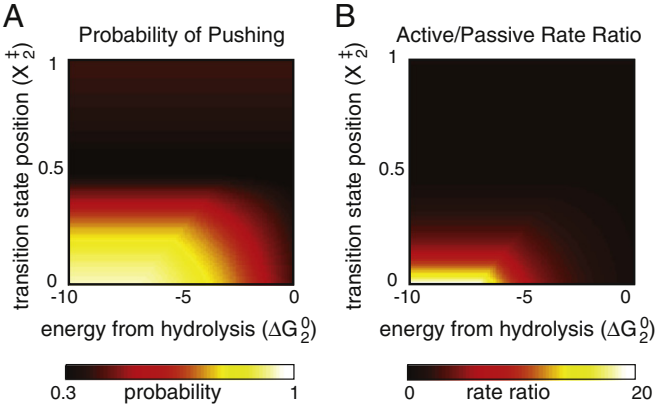

Fig. 3. Effect of polymerase pushing as a function of transition state position of the active enzyme ($x_2^{\ddagger}$) and energy of reaction (ΔG_2^0) of the active enzyme. The passive enzyme parameters are fixed: $x_1^{\ddagger}=0.5$, $\Delta G_1^0=0$, $\Delta G_1^{\ddagger}=1$ $\Delta G_2^{\ddagger}=4$. (A) The probability of the system progressing via pushing. (B) The ratio of the pushing rate and the passive stepping rates.

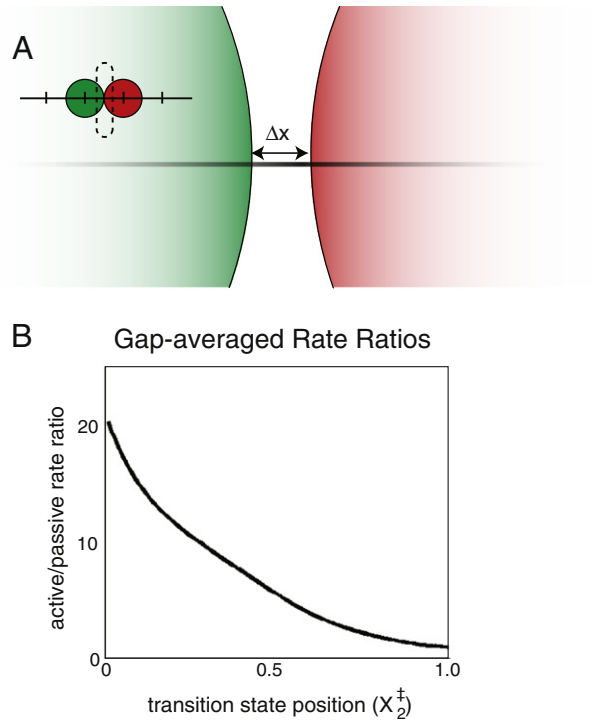

Fig. 4. Effect of polymerase-polymerase gap size on pushing. (A) In general, small (and fluctuating) gaps (Δx) will exist between neighboring polymerases. (B) The average ratio of active and passive rates (k_a/k_p) versus the transition state position of the active enzyme ($x_2^{\ddagger}$) averaged over different values of gap size ($0<\Delta x\leq 1$).

3.3. Enzyme deformation (elasticity in the system)

We have been discussing the case where the polymerases interact as hard spheres. However, recent data suggest that this is not the case when the leading polymerase is unable to move forward due to nucleotide starvation. Under these conditions, the system exhibits elastic deformations [22]. We asked how the transition state position during translocation would affect rates of deformative pushing over the distance of a single base pair. This is the same as asking how translocation rates are affected by force where the force is given by the deformation energy per distance [30]. As early transition states minimize the effect of force in the forward direction, they also lead to accelerated pushing against elastic barriers. This fact underlies previous results from analyses of active and elastic helicase systems [25–27]. We conclude that power stroke mechanisms push more effectively regardless of the elasticity of the enzymes and barriers involved.

4. Conclusion

We have introduced a new pushing mechanism that acts even in the absence of enzyme elasticity. This mechanism is the result of a saddle trajectory that arises in the two-dimensional energy landscape of two interacting enzymes. However, the existence of this trajectory is subject to constraints on the energy landscapes of the individual particles. Specifically, only in cases where the transition state of the active particle is relatively early during a step will pushing occur. This mechanism and previously described elastic mechanisms [25–27] both suggest that the ability of actively elongating polymerases to push backtracked enzymes is sensitively dependent on the position of the translocation transition state. Only when this transition state is early along the reaction path, as it would be in a power stroke-type mechanism, does pushing become favorable. Given that experimental results have observed cumulative pushing effects when multiple enzymes are on the DNA track, we suggest that the energy from NTP hydrolysis is released relatively early during a polymerase step at least in cases when two enzymes interact.

The formalism presented here is applicable to other motor proteins that walk on linear templates with high processivity and displace passive barriers [31]. The results suggest that although both Brownian ratchets and power stroke motors may translocate effectively under loads, power stroke mechanisms lead to an increased ability to directly exert forces on barriers to translocation.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.bpc.2011.04.009.

References

[1] E.A. Abbondanzieri, et al., Direct observation of base-pair stepping by RNA polymerase, Nature 438 (2005) 460–465.
[2] G. Bar-Nahum, et al., A ratchet mechanism of transcription elongation and its control, Cell 120 (2005) 183–193.
[3] P. Cramer, D.A. Bushnell, R.D. Kornberg, Structural basis of transcription: RNA polymerase II at 2.8 angstrom resolution, Science. 292 (2001) 1863–1876.
[4] N.R. Forde, et al., Using mechanical force to probe the mechanism of pausing and arrest during continuous elongation by *Escherichia coli* RNA polymerase, Proc Natl Acad Sci USA 99 (2002) 11682–11687.
[5] J. Gelles, et al., Single-molecule kinetic studies on DNA transcription and transcriptional regulation, Biophys. J. 68 (1995) 73S.
[6] G.A. Kassavetis, M.J. Chamberlin, Pausing and termination of transcription within the early region of bacteriophage T7 DNA in vitro, J. Biol. Chem. 256 (1981) 2777–2786.
[7] I. Sidorenkov, N. Komissarova, M. Kashlev, Crucial role of the RNA:DNA hybrid in the processivity of transcription, Mol. Cell 2 (1998) 55–64.
[8] R. Landick, The regulatory roles and mechanism of transcriptional pausing, Biochem. Soc. Trans. 34 (2006) 1062–1066.
[9] I. Toulokhonov, et al., A central role of the RNA polymerase trigger loop in active-site rearrangement during transcriptional pausing, Mol. Cell 27 (2007) 406–419.
[10] E.A. Galburt, et al., Backtracking determines the force sensitivity of RNAP II in a factor-dependent manner, Nature 446 (2007) 820–823.
[11] J. Shaevitz, et al., Backtracking by single RNA polymerase molecules observed at near-base-pair resolution, Nature 426 (2003) 684–687.
[12] N. Komissarova, M. Kashlev, Transcriptional arrest: *Escherichia coli* RNA polymerase translocates backward, leaving the 3′ end of the RNA intact and extruded, Proc Natl Acad Sci USA 94 (1997) 1755–1760.
[13] M. Voliotis, et al., Fluctuations, pauses and backtracking in DNA transcription, Biophys. J. 94 (2008) 334–348.
[14] M. Kireeva, et al., Nature of the nucleosomal barrier to RNA polymerase II, Mol. Cell 18 (2005) 97–108.
[15] M. Depken, E.A. Galburt, S.W. Grill, The origin of short transcriptional pauses, Biophys. J. 96 (2009) 2189–2193.
[16] S. Klumpp, T. Hwa, Stochasticity and traffic jams in the transcription of ribosomal RNA: intriguing role of termination and antitermination, Proc Natl Acad Sci USA 105 (2008) 18159–18164.
[17] K. Sneppen, et al., A mathematical model for transcriptional interference by RNA polymerase traffic in *Escherichia coli*, J. Mol. Biol. 346 (2005) 399–409.
[18] T. Tripathi, D. Chowdhury., Interacting RNA polymerase motors on a DNA track: effects of traffic congestion and intrinsic noise on RNA synthesis, Phys. Rev. E Stat. Nonlin. Soft Matter Phys. 77 (2008) 011921.
[19] B. Derrida., An exactly soluble non-equilibrium system: the asymmetric simple exclusion process, Physics Reports. 301 (1998) 65–83.
[20] V. Epshtein, E. Nudler, Cooperation between RNA polymerase molecules in transcription elongation, Science. 300 (2003) 801–805.
[21] V. Epshtein, et al., Transcription through the roadblocks: the role of RNA polymerase cooperation, EMBO J. 22 (2003) 4719–4727.
[22] H. Saeki, J.Q. Svejstrup, Stability, flexibility, and dynamic interactions of colliding RNA polymerase II elongation complexes, Mol. Cell 35 (2009) 191–205.
[23] J. Jin, et al., Synergistic action of RNA polymerases in overcoming the nucleosomal barrier, Nat. Struct. Mol. Biol. 17 (2010) 745–752.
[24] S. Proshkin, et al., Cooperation between translating ribosomes and RNA polymerase in transcription elongation, Science. 328 (2010) 504–508.

[25] M.D. Betterton, F. Jülicher, A motor that makes its own track: helicase unwinding of DNA, Phys. Rev. Lett. 91 (2003) 258103.
[26] M.D. Betterton, F. Jülicher., Opening of nucleic-acid double strands by helicases: active versus passive opening, Phys. Rev. E Stat. Nonlin. Soft Matter Phys. 71 (2005) 011904.
[27] A. Garai, D. Chowdhury, M.D. Betterton., Two-state model for helicase translocation and unwinding of nucleic acids, Phys. Rev. E Stat. Nonlin. Soft Matter Phys. 77 (2008) 061910.
[28] P. Hänggi, P. Talkner, M. Borkovec., Reaction-rate theory: fifty years after Kramers, Reviews Modern Physics. 62 (1990) 251–341.
[29] H.A. Kramers, Brownian motion in a field of force and the diffusion model of chemical reactions, Physica. 7 (1940) 284–304.
[30] C. Bustamante, et al., Mechanical processes in biochemistry, Annu. Rev. Biochem. 73 (2004) 705–748.
[31] M. Honda, et al., Single-molecule analysis reveals differential effect of ssDNA-binding proteins on DNA translocation by XPD helicase, Mol. Cell 35 (2009) 694–703.