Published in final edited form as:

Phys Rev E Stat Nonlin Soft Matter Phys. 2013 October ; 88(4): 040703.

Binding-rebinding dynamics of proteins interacting non-specifically with a long DNA molecule

Azita Parsaeian and Monica Olvera de la Cruz

Department of Materials Science and Engineering, Northwestern University, Evanston, Illinois, 60208, USA

John F. Marko

Department of Physics and Astronomy and Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, Illinois, 60208, USA

Abstract

We investigate how non-specific interactions and unbinding-rebinding events give rise to a length- and conformation-dependent enhancement of the “macroscopic” dissociation time of proteins from a DNA, or in general for release of ligands initially bound to a long polymer. By numerically simulating release of ligands from polymers of different conformations, we show that the total dissociation time increases with polymer length logarithmically for an extended conformation, and as a power law for self-avoiding and compact conformations. For the latter two cases, the presence of self-avoidance acting between the diffusing ligands affects the power-law exponents. Our results are important in relating kinetic measurements of protein on- and off- rates for large DNAs to equilibrium affinities for a single binding site.

Many processes in cells are linked to association and dissociation of proteins to and from DNA [1, 2]. The kinetics of binding and unbinding of proteins directly control chromosome structure and function. Many studies have analyzed mechanisms of proteins finding their specific binding sites, and have implicated non-sequence-specific binding in search mechanisms [3–5]. However, much less attention has been paid to analysis of how nonspecific interactions affect the off-kinetics of proteins from large DNA molecules. The stability of any protein-DNA structure depends on the rates of protein binding and unbinding, and their interplay with the conformational relaxation time of the underlying DNA. The dynamics of release of a protein from DNA in most cases will involve a number of rapid unbinding and rebinding events before the protein is able to escape from the region of DNA it was originally bound to; this sequence of rebinding events is in turn dependent on the conformation of the DNA.

In this study, we analyze the effect of rebinding on macroscopic-off rates using a simple simulation model of ligand binding to a long polymer with many equivalent binding sites. Here, “macroscopic” refers to escape of a ligand from one polymer molecule; most assays (*e.g.*, surface-plasmon-resonance (SPR) measurements, many single-molecule fluorescence kinetic experiments) monitor macroscopic dissociation, rather than underlying microscopic on-off events. We then show how the net macroscopic off-time - essentially the time necessary for roughly half the proteins to leave the polymer - depends on the polymer's conformation.

There are a wide range of timescales involved in release of proteins initially bound to a long DNA. Breaking the array of noncovalent bonds holding a protein to DNA can be expected to be slow compared to the “attempt frequency” of about 10^9 sec^{-1} for dissociation of a few-nm-sized protein (*i.e.*, the self-diffusion time of a nm-sized object), due to the energy barrier opposing bond-breaking. Given a barrier of the order of $10k_B T$ “microdissociation” of a

protein from a DNA, involving release of at least some of the chemical interaction holding it to its binding site, is likely to take place at rates of about 10^5 sec^{-1} [6]. Such microdissociation events are likely to occur over a wide range of timescales, and most such events are likely to lead to rebinding of a protein back to a position on the DNA near to that that it started from [7]. This effect may contribute to very slow macroscopic off-rates seen in some protein-DNA interaction experiments (as low as $k_{\text{off,macro}} = 10^{-3} \text{ sec}^{-1}$ [8]).

Here we study the microdissociation-rebinding-macrodissociation process using a simple three-dimensional lattice model where the DNA is represented as a polymer chain and where the ligands (and the binding sites along the DNA) occupy one lattice site. We consider the case where the chain is dissolved in a good solvent, that is, where the monomers repel each other. Hard-core repulsions are defined as excluding conformations where two monomers occupy the same lattice site. We study stretched, swollen and confined chain conformations. Our model is highly coarse-grained; no molecular details or explicit solvent molecules are included, and our emphasis is on extracting scaling laws. While our computations are motivated by the study of DNA-protein interactions, our model can be applied to any ligand that is able to bind at a series of binding sites on a long polymer substrate.

Our analysis is focused on the case where initially, all the binding sites along the long polymer are bound by the small “protein” ligands, in initially ligand-free solution. Thus we effectively consider a “concentration quench” from initially high concentration to have saturated binding, to zero concentration, as might be achieved with DNAs tethered to the inside of a flow cell by simply replacing the protein solution with protein-free buffer. This type of experiment is commonly used to study unbinding rates for protein-DNA interactions (*i.e.*, SPR monitoring of proteins bound to short DNAs [9]), and is also used in single-molecule studies of protein-DNA interactions [10]. Following such a concentration quench, the ligands will dissociate and possibly re-associate with the polymer, but will eventually leave the chain to protein-free solution.

In our simulations, which start with polymer fully saturated by ligands in protein-free solution, we count the number of re-bindings per ligand to the polymer chain. The total number of these revisit events is just proportional to the total time spent by each ligand bound to the polymer; the proportionality constant is just the average microscopic off-time for the ligands. Expressing our results in terms of the number of revisits allows us to study scaling laws connected with rebinding in a microscopic dissociation-time-independent way.

Stretched polymer

We first consider an extended DNA molecule, as might be studied in a single-molecule stretching experiment (Fig. 1) [10]. The unit length a in our simulation is the ligand and binding site (monomer) size, of order a few nm. The unit time τ is the diffusion time for a ligand in solution to move an elementary length, of order 10^{-9} sec. We take the microdissociation time for a bound ligand to be 1000τ , providing well-separated diffusion and dissociation timescales.

Dissociated ligands undergo 3D diffusion, and can re-encounter and rebind, or *revisit* the polymer. We quantify the average macroscopic binding lifetime of the proteins by computing the average number of revisits. We continue the simulation up to a time when the number of revisits (Re) remain constant in time. This timescale is easily identified, and corresponds to the regime where the particles have diffused far enough from the chain that they will not re-encounter it (Fig. 2, inset, shows an example cumulative revisit trace for a 100-monomer polymer). Since each ligand undergoes Brownian motion in the solution, we expect the revisits to occur over the timescale needed for them to move approximately $L =$

Na away from the extended polymer. Given a diffusion constant D for the ligands in solution ($D \approx a^2/\tau$), the revisits occur on a time scale of about L^2/D . The simulations were run up to a time of the order of at least $100L^2/D$, sufficient to calculate the total number of revisits per ligand accurately (for $t = 100L^2/D$, the mean distance of a ligand from the center of the molecule is $r \approx \sqrt{600}L \approx 25L$, and will experience a negligible number of additional re-encounters $\Delta \mathcal{N}_{\text{revisits}} < 2r/L \approx 0.08$ [11]).

Fig. 2 shows the average number of revisits per ligand ($Re/N = \mathcal{N}_{\text{revisits}}$) as a function of polymer length. We observe a slow increase with polymer length which fits well to a logarithmic dependence. Our results in this case are averaged over a chain-length-dependent number of independent runs, from 1000 runs for length $N = 10$ to 50 runs for length $N = 1800$. We have observed the same logarithmic scaling in simulations with and without excluded-volume interactions acting between the diffusing ligands; Fig. 2 shows the result where there are excluded volume interactions between ligands.

The logarithmic behavior can be understood by noting that the rebinding can be considered to count returns to the origin for *two-dimensional* diffusion in the plane perpendicular to the polymer (Fig. 1). The distribution of the unbound ligands in this plane is therefore:

$$\rho(x, y, t) = \frac{1}{4\pi Dt} e^{-(x^2+y^2)/(4Dt)} \quad (1)$$

The weight of this distribution at the origin, which provides the probability of an unbound

ligand rebinding, has the power-law form $\rho(0, 0, t) \sim \frac{1}{Dt}$. Rebinding will occur until diffusion along the polymer axis moves the ligand by a distance of the order of L , that is, over a time scale approximately L^2/D ; the number of rebinding events follows as

$$\mathcal{N}_{\text{revisits}} \approx a^2 \int_{a^2/D}^{L^2/D} dt \rho(0, 0, t) \approx \ln(L/a) \quad (2)$$

We note that a complete description of the dynamics should include dynamic equilibrium of the unbound ligands near the molecule with the ligands which are actually bound (at $(x, y) = (0, 0)$); however, the scaling behavior of Eq. 2 arises from the large- L and therefore long-time limits, in which the bound ligand fraction is small, allowing the use of the “free” diffusion propagator (Eq. 1).

Self avoiding walk (SAW)

We now consider the same polymer, but in a “frozen” SAW configuration (Fig. 3 inset), generated using a pivot algorithm [12, 13]. The frozen conformation reflects a case where ligand dissociation and diffusion are rapid compared to the polymer conformational fluctuations. The inset of the top panel of Fig. 3 shows the radius of gyration R_g versus chain length, which follows the expected SAW power law $R_g = N^\nu$ with exponent $\nu = 0.5901 \pm 0.002$ ($\approx 3/5$), *i.e.*, the expected SAW scaling behavior [14]. We then used the frozen SAW configurations to compute the number of revisits, starting from a fully-occupied configuration as in the stretched polymer case. Our results are averaged over up to 100 configurations (one realization per configuration) with longer polymers averaged over 50 configurations.

The result, shown in the top panel of Fig. 3 for the case with ligand-ligand excluded volume, shows a power-law dependence on SAW length, $\mathcal{N}_{\text{revisits}} \approx N^\alpha$ with $\alpha = 0.475 \pm 0.005$. Without ligand excluded volume, a smaller exponent is observed, $\alpha = 0.41 \pm 0.01$ (Fig. 3, lower panel). Although the SAW is much smaller in overall size than the stretched chain, in

both cases, the number of revisits increase much more strongly with polymer length, corresponding to a stronger dependence of macroscopic off-time on polymer length.

A Flory-like estimate of the expected revisit number for the SAW can be made using the average monomer density in the SAW, $\phi \approx N/R_g^3$. Since the ligands move by diffusion, they will collide with $n \approx R_g^2/a^2$ monomer-sized sites as they move away from the SAW. Thus we can expect $\mathcal{N}_{\text{revisits}} \approx n\phi N^{1-\nu}$, giving $\alpha_{\text{Flory}} = 1 - \nu$. This matches the exponent for the case without ligand-ligand self avoidance ($\alpha \approx 0.41$, Fig. 3 lower panel), but is significantly below the exponent including ligand-ligand self-avoidance ($\alpha \approx 0.48$, Fig. 3 upper panel). Thus, the ligand-ligand excluded volume shifts the exponent α to a value larger than α_{Flory} ; exclusion interactions between the diffusing ligands *increase* the number of re-encounters between them and the SAW.

Confined chain

As a third case we consider a polymer of length N that is collapsed by confinement in a sphere of radius R . We imagine the confining sphere to be permeable, in the sense that the ligands are able to escape and come back. For example, one might consider a chromosomal DNA confined inside a cell nucleus, where the ligands are proteins which interact with DNA but which are able to pass through nuclear pores to the cytoplasm. We adjust R for different N so as to keep the average monomer density fixed at $\phi = 3Na^3/(4\pi R^3) = 0.06$. This constraint gives chains with compact scaling, $R \approx L^\nu$ with $\nu = 1/3$. Again, we consider a frozen polymer configuration where the monomers are fully occupied at the start of the simulation, and we consider the influence of ligand-ligand excluded volume. Results are averaged over 50 configurations, again with one realization per configuration for each polymer length.

Our results for the average $\mathcal{N}_{\text{revisits}}$ for varied N , with ligand-ligand excluded volume, are shown in Fig. 4. The inset of Fig. 4 shows the result where the ligands move with no excluded volume interactions. Both cases show power-law behavior $\mathcal{N}_{\text{revisits}} \approx N^\alpha$ with $\alpha = 0.61 \pm 0.01$ in the case with ligand-ligand excluded volume, and $\alpha = 0.67 \pm 0.01$ when there is no ligand-ligand excluded volume. Compared to the SAW case, the compact confined chains are revisited by escaping ligands much more frequently; chain confinement increases the macroscopic off-time of the ligands.

The Flory estimate $\mathcal{N}_{\text{revisits, Flory}} \approx N^{\alpha_{\text{Flory}}}$ again applies, but now $\nu = 1/3$, giving $\alpha_{\text{Flory}} = 2/3 \approx 0.66$. This matches the simulation result without ligand-ligand excluded volume, but is larger than the exponent for the case including excluded volume interactions between the ligands. Thus, in the compact polymer case, the presence of excluded-volume interactions between the ligands *decreases* the exponent below the Flory estimate, the opposite of the effect we have observed for the SAW case.

To summarize, we have studied the relation between microscopic and macroscopic dissociation of ligands from a long polymer, and we have found that the total time that a ligand is bound depends on the length and conformation of the polymer, due to changes in the scaling of $\mathcal{N}_{\text{revisits}}$. In general we have found $\mathcal{N}_{\text{revisits}} \approx N^\alpha$, with an exponent which has a Flory estimate of $\alpha = 1 - \nu$. For a stretched polymer ($\nu = 1$) we find logarithmic behavior ($\mathcal{N}_{\text{revisits}} \ln N$). For both SAW and confined cases we find power law scaling, $\mathcal{N}_{\text{revisits}} \approx N^\alpha$. When the ligands move without excluded volume, the exponents are very close (within our statistical errors) of the Flory estimate, $\alpha = 1 - \nu$. However, in the presence of ligand-ligand excluded volume, the exponents are shifted from the Flory estimates.

The shifts of revisit exponents away from their Flory values indicate that correlations in the positions of the moving ligands can change the rate at which they escape from their binding substrate. In the SAW case, ligand-ligand self-avoidance *increases* the number of revisits, while in the confined polymer case, ligand-ligand self-avoidance *decreases* the number of revisits. Considering only the effect of crowding in the “atmosphere” of released ligands, one might expect that adding self-avoidance would increase the number of revisits in both cases. However, there is a competition in the chemical potentials experienced by the ligands, between the gradient in the concentration of diffusing molecules and a virial term. We examined the density profiles of the ligands from the center of mass in their initial state, and when half of the ligands have dissociated from the polymer. We see a sharp gradient in ligand concentration in the collapsed chain (at both initial and later times), whereas for the SAW case the density profile decreases smoothly. For the collapsed chain, the sharp ligand concentration profile blocks re-entry of released ligands to the polymer, leading to fewer rebinding events than expected from the Flory estimate. It would be interesting to test this exponent-shift effect experimentally. Quantitative calculation of the exponent shifts resulting from ligand-ligand excluded volume and their dependence on statistical of the polymer binding substrate presents a new and challenging nonequilibrium statistical mechanics problem.

Recent experiments [8] indicate that the kinetics of release of proteins from a DNA can be much slower than one might expect from apparent binding affinities. One contributor to this effect may be the fact that microscopic dissociation from a particular binding site is quite likely to be followed by rebinding to a nearby binding site along the same DNA molecule, leading to an increase in the apparent “off-time” from the DNA. We emphasize that in the experiments of Ref. [8], the proteins showed a strong dependence of off-rate on concentration of protein in solution, combined with an appreciable fraction of proteins which simply did not dissociate to protein-free solution on the time scale of the experiment. Our model does not describe these effects, which most likely require addition of additional, nonclassical “protein exchange” kinetic processes [8].

However, the same general type of experiment as used in Ref. [8], applied to proteins which show concentration-independent off-rates, or at least complete dissociation at zero solution-phase protein concentration, should be able to probe the length- and conformation-dependent dissociation kinetics discussed in this paper. We emphasize that if one attempts to estimate a chemical affinity using off-rate data in dilute solution, one may infer a molecule length- or conformation-dependent affinity. Similarly, the effective dissociation time for a protein from a chromosomal site in vivo will likely depend on the local structure and conformation of the chromosome, with apparent off-rates that are quite different for stretched or isolated DNAs, from those for a DNA confined to a region of the cell nucleus [15] or bacterial nucleoid [2].

Acknowledgments

This research was supported by the NSF through Grant Nos. DMR-0907781, DMR-1206868 and MCB-1022117 and by the NIH through Grants No. 1U54CA143869 (NU-PS-OC) and No. R01GM105847. MO thanks the DOD NSSEFF award, AFOSR Award FA9550-10-1-0167, for computational support.

References

- [1]. Dame RT, Kalmykova OJ, Grainger DC. PLoS Genet. 2011; 76:e1002123. [PubMed: 21698131]
- [2]. Rimsky S, Travers A. Curr. Opin. Microbiol. 2011; 14(2):136–141. [PubMed: 21288763]
- [3]. Halford SE, Marko JF. Nucleic Acids Res. 2004; 32:3040–3052.
- [4]. Halford SE, Szczelkun MD. Eur. Biophys. J. 2002; 31:257–267. [PubMed: 12122472]

- [5]. von Hippel PH, Berg OG. J. Biol. Chem. 1989; 264:675–678. [PubMed: 2642903]
- [6]. Kuznetsov SV, Kozlov AG, Lohman TM, Ansari A. J. Mol. Biol. 2006; 359:55–65. [PubMed: 16677671]
- [7]. Loverdo C, Benichou O, Voituriez R, Biebricher A, Bonnet I, Desbailles P. Phys. Rev. Lett. 2009; 102:188101. [PubMed: 19518914]
- [8]. Graham JS, Johnson Reid. C. Marko JF. Nucleic Acids Res. 2010; 39:2249.
- [9]. Brockman JM, Frutos AG, Corn RM. J. Am. Chem. Soc. 1999; 121:8044–8051.
- [10]. Xiao B, Zhang H, Johnson RC, Marko JF. Nucleic Acids Res. 2010; 38:6176–85.
- [11]. Berg, HC. Random Walks in Biology. Princeton, Princeton: 1993. Ch. 3
- [12]. Lal M. Molec. Phys. 1969; 17(4):57–64.
- [13]. Madras N, Sokal AD. J. Stat. Phys. 1988; 50:109–186.
- [14]. de Gennes, PG. Scaling Concepts in Polymer Physics. Cornell University Press; 1985.
- [15]. Cremer T, Cremer M, Dietzel S, Muller S, Solovei I, Fakan S. Curr. Opin. Cell Biol. 2006; 18:307–16. [PubMed: 16687245]

FIG. 1.

A protein undergoes a number of unbinding-rebinding events before it diffuses away to the bulk solution. Projection of 3D diffusion trajectory into plane perpendicular to extended DNA; revisits correspond to re-encounters with the origin in the two-dimensional plane.

FIG. 2.

Semi-Log plot of the number of protein revisits per ligand ($Re/N = \mathcal{N}_{\text{revisits}}$) is proportional to the logarithm of the length of the chain. The straight line is a logarithmic fit to $\mathcal{N}_{\text{revisits}} = a \ln(N) + b$ with fitting parameters $a = 0.73 \pm 0.02$ and $b = -0.35 \pm 0.09$. *Inset:* Total number of revisits vs. time; we continue the simulation long enough to reach a constant total number of revisits.

FIG. 3.

Top panel: Log-log plot of number of protein revisits per ligand vs. length of SAW chain ($Re/N = \mathcal{N}_{\text{revisits}}$), for calculations including ligand-ligand excluded volume. The straight line is a fit to $\mathcal{N}_{\text{revisits}} = a N^\alpha$ with fitting parameters $\alpha = 0.475 \pm 0.005$ and $a = 0.72 \pm 0.01$.

Inset: Scaling of the R_g for free SAW polymer chains, for chains $100 \leq N \leq 1000$, log-log plot. The straight line is a fit to $R_g = b N^\nu$ with fitting parameters $\nu = 0.5914 \pm 0.0002$ and $b = 1.070 \pm 0.001$.

Bottom panel: Log-log plot of number of revisits of ligands to DNA per DNA site with no ligand-ligand excluded volume interactions. The straight line is a fit to $\mathcal{N}_{\text{revisits}} = a_1 N^{\alpha_1}$ with fitting parameters $\alpha_1 = 0.41 \pm 0.01$ and $a_1 = 0.48 \pm 0.05$. *Inset:* Sample simulation result of a SAW polymer configuration with $N = 600$.

FIG. 4.

Log-log plot of revisits per diffusing ligand ($Re/N = \mathcal{N}_{\text{revisits}}$) vs. chain length for a polymer confined in a sphere with density $\phi = 0.06$, with ligand-ligand excluded volume interactions. The straight line is a fit to $\mathcal{N}_{\text{revisits}} = a N^\alpha$ with fitting parameters $\alpha = 0.61 \pm 0.01$ and $a = 0.38 \pm 0.02$. *Inset:* Results for equivalent simulations but without ligand-ligand excluded volume. The straight line is a fit to $\mathcal{N}_{\text{revisits}} = a_1 N^{\alpha_1}$ with fitting parameters $\alpha_1 = 0.67 \pm 0.01$ and $a_1 = 0.20 \pm 0.01$.