Subdiffusion and weak ergodicity breaking in the presence of a reactive boundary

Michael A. Lomholt,1 Irwin M. Zaid,2 and Ralf Metzler3,1

1Physics Department, University of Ottawa, Pavillon MacDonald, Ottawa, Ontario K1N 6N5, Canada
2Physics Department, Carleton University, Herzberg Building, Ottawa, Ontario K1S 5B6, Canada
3Physics Department, Technical University of Munich, 85748 Garching, Germany

We derive the boundary condition for a diffusive particle interacting with a reactive boundary with finite reaction rate. Molecular crowding conditions, that are found to cause subdiffusion of larger molecules in biological cells, are shown to effect long-tailed distributions with identical exponent for both the unbinding times from the boundary to the bulk and the rebinding times from the bulk. This causes a weak ergodicity breaking: typically, an individual particle either stays bound or remains in the bulk for very long times. We discuss why this may be beneficial for in vivo gene regulation by DNA-binding proteins, whose typical concentrations are nanomolar.

PACS numbers: 05.40.Fb,02.50.Ey,82.20.-w,87.16.-b

The interaction of a diffusive particle with a reactive boundary is of fundamental importance in interface science and technology, e.g., to transport in porous media, interactions of proteins with artificial surfaces and membranes, or applications such as foam relaxation and surfactants. For a Brownian particle this has been studied extensively, especially concerning the question how bulk exchange influences the surface distribution of intermittently adsorbed particles. Here, we derive the exchange dynamics with a reactive boundary of a subdiffusing particle, whose unbinding and rebinding times in a molecular crowding environment are both shown to follow long-tailed distributions. We demonstrate a weak ergodicity breaking for the particle trajectory.

This is of particular interest for the search of DNA-binding proteins for their specific binding site on DNA involving successive events of non-specific binding to the DNA and bulk excursions, such that the time spent in either of these events is important in the understanding of the various stochastic mechanisms involved in (bacterial) gene regulation. While the generally applied assumption of Brownian diffusion of proteins works well for typical in vitro experiments under dilute conditions, in vivo the abundance of a multitude of biomacromolecules in the cellular cytoplasm have been shown to cause a state of molecular crowding: large molecules such as proteins, lipids, RNA molecules and ribosomes make up to 40% of the cytoplasmic volume. In this superdense environment they hinder each other’s motion, causing subdiffusion, with a mean squared displacement \( \langle r^2(t) \rangle \propto t^\alpha \), where \( 0 < \alpha < 1 \). Subdiffusion was verified for proteins in membranes with \( \alpha \approx 0.7 \), for proteins in a molecular crowded in vitro environment with \( \alpha \approx 0.75 \) at higher densities, and as well as in the cytoskeleton in vivo for messenger RNA of physical size \( \approx 100 \text{nm} \) with \( \alpha \approx 0.75 \) and for dextran molecules ranging from 10kD to 2 MD with \( \alpha \) in between 0.59 and 0.84. The occurrence of subdiffusion for particles with mass as low as 10kD was also confirmed by computer simulations.

The Lac repressor, a typical DNA-binding protein, has 141kD for which a corresponding \( \alpha \approx 0.73 \) was found. This, under molecular crowding conditions, \( \alpha \approx 0.75 \) seems a fairly standard value for DNA-binding proteins and larger polynucleotides. The time scale over which this subdiffusion persists is not known precisely, but appears to be longer than minutes, so that the following considerations are expected to be relevant for genetic processes.

To derive the generalized reactive boundary condition, we pursue a continuous time random walk approach similar to Ref. [10]: A subdiffusing particle jumps from one point to the next after a waiting time distributed according to the long-tailed probability density \( \psi(t) = t^{\alpha}/t^{1+\alpha} \). We start our derivation with the one-dimensional lattice, on which \( A_i \) is the probability to find the particle at lattice point \( i = 1, 2, 3, \ldots \). The probability of being at the reactive site (lattice point next to the boundary) is \( A_0 \), the notation indicating that at site 0 the particle can be exchanged with the bound state with rate \( \kappa \). The balance equations then read

\[
\begin{align*}
\frac{dA_i(t)}{dt} &= \frac{i}{\langle r^2(t) \rangle} A_i(t) - \frac{(i-1)}{\langle r^2(t) \rangle} A_{i-1}(t), \quad (1a) \\
\frac{dA_0(t)}{dt} &= \frac{i}{\langle r^2(t) \rangle} A_0(t) - \kappa A_i(t), \quad (1b)
\end{align*}
\]

and the loss from a given lattice site due to diffusion is

\[
\begin{align*}
I_i(t) &= \psi(t)A_i(0) + \int_0^t \psi(t-t')I_i(t')dt', \quad (2a) \\
I_0(t) &= \psi_\kappa(t)A_0(0) + \int_0^t \psi_\kappa(t-t')I_0(t')dt', \quad (2b)
\end{align*}
\]

where \( \psi_\kappa(t) \equiv \psi(t)e^{-\kappa t} \). Substituting for \( I^* \) from Eqs. [1], we rephrase Eqs. [2] in the form \( I_i(t) = \int_0^t \Phi(t-t')A_i(t')dt' \). The kernel \( \Phi(t) \) is defined by \( \Phi(u) = \psi(u)/[1 - \psi(u)] \) in the Laplace domain, \( \Phi(u) = \int_0^\infty \Phi(t)e^{-ut}dt \). An analogous relation holds for \( I_0(t) \), with the kernel \( \Phi_\kappa(u) = \Phi(u + \kappa) \). For the gain to site \( i \) we have, assuming that the particle jumps to left and right equally likely, \( I_i^* = I_{i-1}/2 + I_{i+1}/2 \) and...
the sought for reactive boundary condition
\[ P(0) = \frac{\kappa}{\kappa + \kappa_{\text{off}}} e^{-\kappa_{\text{off}} t}. \]
The points are results from a stochastic simulation [22].

Collecting the results, we obtain for \( \mathcal{P} \), where in the continuum limit (\( \kappa \to 0 \)) we have \( \kappa \ll \kappa_{\text{off}} \). The unbinding times then are distributed according to the power-law \( \mathcal{P}_{\text{unb}} \sim 1/(\kappa_{\text{off}} t^{1+\alpha}) \). This is a central finding of this work: The crowded environment impeding the desorption to the bulk translates the a priori exponential distribution of unbinding times to a power-law [21]. Once arrived at site \( i = 1 \), the particle subdiffuses in the bulk. We consider here the cylindrical case governed by Eq. [10]. With initial condition \( P_0 = 1 \) and a reflecting boundary condition at \( r = R_2 \), an analytic result can be obtained in terms of modified Bessel functions, see Ref. [22] for details. A systematic expansion for small \( u \) leads to the result [22]

\[
\psi_{\text{reb}}(u) \sim 1 - Su^{\alpha}/k_{\text{on}}.
\] (17)

with the cylindrical cross-section \( S = \pi (R_2^2 - R_1^2) \). The form \( \psi_{\text{reb}}(t) \sim t^{-1-\alpha} \) is typical for subdiffusion [14].

Both unbinding to the volume and returning to the reactive boundary follow power-law forms with identical asymptotic behavior \( \sim t^{-1-\alpha} \). The lack of a characteristic time scale separating micro- and macroscopic events gives rise to weak ergodicity breaking [23]. As shown in Ref. [24], the time-averaged probability in the bound state \( \overline{\mathcal{P}}_{\text{bound}} = \lim_{t \to \infty} \mathcal{P}_{\text{bound}}/t \) for a single trajectory, \( \overline{\mathcal{P}}_{\text{bound}} \) has the distribution \( \mathcal{P}(\overline{\mathcal{P}}_{\text{bound}}) = \delta_\alpha \left( k_{\text{on}}/(S k_{\text{off}}) \right) \overline{\mathcal{P}}_{\text{bound}} \), with the Lamperti-generalized \( \delta \)-function [24, 25]

\[
\delta_\alpha(\beta, \rho) = \frac{\pi^{-1} \sin(\pi \alpha) \beta \rho^{\alpha-1}(1-\rho)^{\alpha-1}}{\beta^{2}\rho^{2\alpha} + \rho^{2\alpha}(1-\rho)^{\alpha} \cos \pi \alpha}.
\] (18)

Note that \( \mathcal{P} \) is normalized, \( \int_0^1 \mathcal{P}(\overline{\mathcal{P}}_{\text{bound}})\overline{\mathcal{P}}_{\text{bound}} \text{d}\overline{\mathcal{P}}_{\text{bound}} = 1 \), and valid in the long time limit. It is independent of \( t \) and in that sense an equilibrium is attained. However, while in the Brownian limit \( \alpha = 1 \), ergodicity and a sharply peaked behavior for \( \mathcal{P} \) are recovered, the very distinct behavior of \( \mathcal{P} \) for \( \alpha < 1 \) is displayed in Fig. for \( \alpha = 0.75 \): as function of \( \rho = \overline{\mathcal{P}}_{\text{bound}} \), the distribution peaks at 0 and 1, with a smaller maximum in between. Thus, in a single trajectory a particle is typically either bound or unbound, independently of the duration of the trajectory. This nonergodic behavior is imposed on the system by the probability \( \int_t^\infty \psi(t')dt' \sim t^{-\alpha} \) of never moving, that decays very slowly. The smaller the cross section \( S \), the more likely is it to find the particle in a bound state, as it should be. The behavior of \( \mathcal{P} \) therefore contrasts the ensemble average over many trajectories, \( \langle \overline{\mathcal{P}}_{\text{bound}} \rangle = (1 + Sk_{\text{off}}/k_{\text{on}})^{-1} \), corresponding to the form \( \mathcal{P}(\overline{\mathcal{P}}_{\text{bound}}) = \delta(\overline{\mathcal{P}}_{\text{bound}} - k_{\text{on}}/(k_{\text{on}} + Sk_{\text{off}})) \) [24]. This can be understood as follows. For an ensemble of particles, \( k_{\text{on}}/k_{\text{off}} \) defines the nonspecific binding constant \( K_{\text{ns}} \), equal to the ratio \( N_{\text{bound}}/(SN_{\text{unbound}}) \) of bound and unbound particles normalized by the cross section \( S \). Then \( 1/(1+Sk_{\text{off}}/k_{\text{on}}) \) is the ensemble probability that a particle is bound. Weak ergodicity breaking is thus relevant for systems with few particles of a given species.

Transcription factors (TFs), DNA-binding proteins regulating the transcription of a specific gene, occur at very small numbers (a few to some hundred per cell [26]), and in many cases it is essential for the stability of genetic circuits that a TF is always bound at some operational level [28]. There exists a large class of TFs, such as the well-studied Lac and bacteriophage \( \lambda \) repressors in \( E. \ coli \) [28], whose specific binding site is located immediately adjacent to their coding region. Biochemical production occurs likely within a few tens of nm from the coding region [29], and therefore from the targeted binding site. The weak ergodicity breaking thus keeps these TFs within a small volume around their complete

![FIG. 1: The distribution \( \delta_\alpha \), Eq. (15), for various \( \beta \), with \( \alpha = 0.75 \). In all cases, a divergence at \( \rho = 0 \) and 1 is observed. The points are results from a stochastic simulation [22].](image-url)
biochemical cycle, very likely leading to a significant increase in the stability of the regulation of that particular gene. Subdiffusion caused by molecular crowding could therefore be very beneficial for living cells, allowing them to maintain the concentrations of even vital TFs at nanomolar levels. This may significantly impact our current picture of gene regulation in vivo and pose the need to perform experiments much closer to the cellular crowding conditions in order to obtain meaningful information for the in vivo situation.

We derived the generalized reactive boundary condition for the interaction of a subdiffusive particle with a boundary and showed that in the molecular crowding scenario the distribution of unbinding times becomes long-tailed, with the same exponent as the distribution of return times to the boundary. This gives rise to weak ergodicity breaking, relevant for systems with small numbers of diffusing particles. Apart from gene regulation, these effects will impact cellular processes in more general, such as the interactions of biopolymers with membrane proteins, or the exchange of shorter DNA and RNA chains across cellular membranes. Moreover, they will affect trapping phenomena in the vicinity of soft interfaces in more general, e.g., the exchange dynamics from ion clouds in the vicinity of charged or polarized membranes. It should be very interesting to explore these effects by single particle tracking under molecular crowding conditions using fluorescent labelling techniques.

We thank Igor Sokolov and Eli Barkai for helpful discussions, and acknowledge funding by NSERC of Canada and the Canada Research Chairs programme.

[1] S. Stapf, R. Kimmich, R. O. Seitter, Phys. Rev. Lett. 75, 2855 (1995); P. Levitz et al., ibid. 96, 180601 (2006).
[2] L. Vroman, A. L. Adams, G. C. Fischer, and P. C. Munoz, Blood 55, 156 (1980); J. L. Brash et al., ibid. 71, 932 (1988); J. G. Donaldson, R. A. Kahn, J. Lipincott-Schwartz, and R. D. Klausner, Science 254, 1197 (1991).
[3] A. A. Sonin, A. Bonfillon, and D. Langevin, Phys. Rev. Lett. 71, 2342 (1993); C. Stenvert and D. Langevin, Langmuir 4, 1179 (1988).
[4] See, e.g., O. V. Bychuk and B. O’Shaughnessy, Phys. Rev. Lett. 74, 1795 (1995); R. Valuillin, R. Kimmich, and N. Fatkullin, Phys. Rev. E 56, 4371 (1997).
[5] P. H. von Hippel and O. G. Berg, J. Biol. Chem. 264, 675 (1989), and Refs. therein.
[6] M. Slutsky and L.A. Mirny, Biophys. J. 87, 4021 (2004); M. Coppey, O. Bénichou, R. Voituriez, and M. Moreau, Biophys. J. 87, 1640 (2004); I.M. Sokolov, R. Metzler, K. Pant, and M.C. Williams, Biophys. J. 89, 895 (2005); Y. M. Wang, R. H. Austin, and E. C. Cox, Phys. Rev. Lett 97, 048302 (2006).
[7] M. A. Lomholt, T. Ambjörnsson, and R. Metzler, Phys. Rev. Lett. 95, 260603 (2005).
[8] K. Takahashi, S. N. V. Arjunan, and M. Tomita, FEBS Lett. 579, 1783 (2005); S. Zimmermann and A. Minton, Annu. Rev. Biophys. Biomol. Struct. 22, 27 (1993).
[9] R. J. Ellis and A. P. Minton, Nature 425, 27 (2003); G. Rivas, F. Ferrone, and J. Herzfeld, EMBO Rep. 5, 23 (2004); A. B. Fulton, Cell 30, 345 (1982).
[10] M. Weiss, H. Hashimoto, and T. Nilsson, Biophys. J. 84, 4043 (2003).
[11] D. S. Banks and C. Fradin, Biophys J. 89, 2960 (2005).
[12] I. Golding and E.C. Cox, Phys. Rev. Lett. 96, 098102 (2006).
[13] M. Weiss, M. Elsner, F. Karthberg, and T. Nilsson, Biophys. J. 87, 3518 (2004).
[14] R. Metzler and J. Klafter, Phys. Rep. 339, 1 (2000); J. Phys. A 37, R161 (2004).
[15] A. E. Chakerian and K. S. Matthews, J. Biol. Chem. 266, 22206 (1991).
[16] I. M. Sokolov, M. G. W. Schmidt, and F. Sagués, Phys. Rev. E 73, 031102 (2006).
[17] E. W. Montroll and G. H. Weiss, J. Math. Phys. 6, 167 (1965); H. Scher and E. W. Montroll, Phys. Rev. B 12, 2455 (1975). J. Klafter, A. Blumen, and M. F. Shlesinger, Phys. Rev. A 35, 3081 (1987).
[18] We denote the Laplace transform \( f(u) \) of a function \( f(t) \) by explicit dependence on the argument.
[19] We keep \( K_{\alpha} = a^2/(2\pi^2) \), \( \kappa \), and \( \kappa_{\text{off}} \) fixed, implying \( \tau \simeq a^{2/\alpha} \), \( \kappa \simeq a^{-1/\alpha} \), and \( \kappa_{\text{off}} \simeq a^{1-1/\alpha} \).
[20] A different scenario corresponding to exponential unbinding in a trapping environment is discussed in Ref. [22].
[21] Crossing of this boundary is viewed to lead the particle into an equivalent cylinder, see O. G. Berg and C. Blomberg, Biophys. Chem. 4, 367 (1976).
[22] I. Zaid, M. A. Lomholt, and R. Metzler (unpublished).
[23] J.-P. Bouchaud, J. Phys. I (Paris) 2, 1705 (1992); J. Bel and E. Barkai, Phys. Rev. Lett. 94, 240602 (2005).
[24] G. Bel and E. Barkai, Phys. Rev. E 73, 016125 (2006).
[25] J. Lamperti, Trans. Am. Math. Soc. 88, 380 (1958).
[26] P. Guptasarma, BioEssays 17, 987 (1995).
[27] See, e.g., A. Bakk and R. Metzler, FEBS Lett. 563, 66 (2004); J. Theor. Biol. 231, 525 (2004), and Refs. therein.
[28] M. Ptashne, *A Genetic Switch* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2004).
[29] S. Hofmann and O. L. Miller, J. Bacteriol. 132, 718 (1977); P. B. Warren and P. R. ten Wolde, J. Mol. Biol. 342, 1370 (2004).