

**Comments to the Editor. Reply to the Comment by
V.P. Shkilev on "Anomalous versus slowed-down
Brownian diffusion in the ligand-binding equilibrium"**

Hédi Soula, Bertrand Caré, Guillaume Beslon, Hugues Berry

► **To cite this version:**

Hédi Soula, Bertrand Caré, Guillaume Beslon, Hugues Berry. Comments to the Editor. Reply to the Comment by V.P. Shkilev on "Anomalous versus slowed-down Brownian diffusion in the ligand-binding equilibrium". Biophysical Journal, Biophysical Society, 2014, 106 (11), pp.2544-2546. <[http://www.cell.com/biophysj/abstract/S0006-3495\(14\)00453-6](http://www.cell.com/biophysj/abstract/S0006-3495(14)00453-6)>. <10.1016/j.bpj.2014.03.052>. <hal-00956603>

HAL Id: hal-00956603

<https://hal.inria.fr/hal-00956603>

Submitted on 6 Mar 2014

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Comments to the Editor

Reply to the Comment by V.P. Shkilev on “Anomalous versus slowed-down Brownian diffusion in the ligand-binding equilibrium”

Hédi Soula,^{*†‡} Bertrand Caré,^{†‡} Guillaume Beslon,^{†§} and Hugues Berry^{*†§}

[†] EPI Beagle, INRIA Rhône-Alpes F-69603, Villeurbanne, France, [‡] Université de Lyon, Inserm UMR1060 F-69621 Villeurbanne, France, and [§] LIRIS, Université de Lyon UMR 5205 CNRS-INSA, F-69621, Villeurbanne, France

*Correspondence: hedi.soula@insa-lyon.fr or hugues.berry@inria.fr

In his comment in this issue of the *Biophysical Journal*, V.P. Shkilev (1) disagrees with some of the findings of our article on the influence of diffusion on the ligand-binding equilibrium (2). V.P. Shkilev makes two main claims in his comment: (i) when diffusion is uniformly Brownian (with a space-independent diffusion coefficient), “the equilibrium fraction of bound receptors [...] should depend on the diffusion coefficient” and (ii) when molecules undergo transient subdiffusion due to a Continuous-Time Random Walk (CTRW), the equilibrium fraction of bound receptors can only be equal or larger (but not smaller) than the value expected for uniform Brownian motion with comparable diffusion coefficient. While the former is a frequent misconception related to the ligand-binding equilibrium, the latter relates to an important issue in the field of (anomalous) subdiffusion-reaction couplings.

Diffusion-dependence of the ligand binding equilibrium.

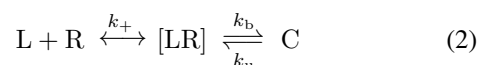
Even when the movement of each molecule is a simple Brownian motion with constant diffusion coefficient, the influence of diffusion on the ligand-binding equilibrium, eq. (1), is non-trivial:



where k_{on} and k_{off} are the global forward and backward reaction rates, respectively. An overview of the impact of Brownian diffusion on this reaction can be found in the book by J. Linderman and D. Lauffenburger (3) (see section 4.2 in their book). We recall below their major results.

The central point is that the classical one step process eq. (1) is actually not a good way to represent this reaction-diffusion process. The ligand-binding equilibrium is in essence a two-step process, that requires first the diffusive transport of individual molecules L and R (with rate constant k_+) until their distance is small enough to allow the

intrinsic binding step to take place:



This two-step process thus defines an “encounter complex” $[LR]$ in which L and R are close enough to bind but have not yet bound. $[LR]$ then can undergo an intrinsic reversible binding reaction with intrinsic reaction rates k_b and k_u , that do not depend on the transport process. In agreement with the celebrated Smoluchovski’s rate law, the transport rate constant (or diffusion-limited rate constant) reads: $k_+ = 4\pi(D_L + D_R)a$ (3, 4), where D_L and D_R are the diffusion coefficients of L and R, respectively, and a is the capture radius. Now, the global forward reaction rate k_{on} depends on the diffusion coefficient through k_+ according to $k_{\text{on}} = k_+k_b/(k_b + k_+)$ (3, 4). Therefore, the global forward constant rate is expected to increase with the diffusion coefficients $D_L + D_R$. This can for instance be seen in figure 2A of our article (2) where the time needed to reach equilibrium increases when the diffusion coefficient (thus k_{on}) decreases.

Now, when applied to the global backward constant rate k_{off} , this approach leads to the remarkable conclusion that the global backward step is not a local process but also depends on diffusivity. This can be illustrated with the following toy example. Consider the case where the diffusion coefficients vanish for all species ($D_L = D_R = D_C = 0$) and the reaction is initiated with only C complexes (no free L nor R initially). In this case, dissociated species L and R cannot appear; the dynamics is restricted to transitions between “real” complexes C and “encounter” complexes $[LR]$. It follows that the global dissociation rate constant $k_{\text{off}} = 0$ in this case, which illustrates the fact that k_{off} too depends on the diffusion coefficient.

Theoretical approaches by e.g. Lauffenburger and Linderman (3), Shoup and Szabo (4) indeed lead to $k_{\text{off}} = \lambda k_u$ where $\lambda = k_+/(k_b + k_+)$ is the escape probability, i.e. the probability that an encounter complex $[LR]$ actually dissociates into free R and L molecules. Since the global associa-

tion rate constant k_{on} has the same dependence on D via k_+ , the dependence on the diffusion coefficient cancels out in the ratio $K_D = k_{\text{off}}/k_{\text{on}}$. Therefore, when molecules move via a (uniform) Brownian motion, the equilibrium fraction of bound receptors $C_{\text{eq}} = L_T R_T / (K_D + L_T)$ (where L_T and R_T are the total concentrations of L and R, respectively) does not depend on the diffusion coefficient, in agreement with standard equilibrium thermodynamics.

Contrarily to the claim by V.P. Shkilev in his comment (1), even for $D = 0.02$ or 0.01 , the equilibrium fraction of bound receptors (at long times) in our simulations with Brownian motion does not depend on the value of D . We agree that this was not obvious from Figure 2A where the reaction times were set so as to evidence the difference in the time needed to reach equilibrium but was not large enough to actually show the equilibrium for the smallest diffusion coefficients. However this result was directly displayed in our Figure 3B1 that shows the equilibrium constant K_D (thus, indirectly, the equilibrium fraction of bound receptors) for Brownian motion with various values of the diffusion coefficient D . This figure clearly evidenced that the equilibrium fraction of bound receptors does not depend on D when molecules move via Brownian motion.

The ligand binding equilibrium with CTRW-based transient subdiffusion.

In our article, transport by CTRW-based transient subdiffusion is obtained when the distribution of waiting times (between two movements) is given by the power law $\phi(\tau) = \alpha \tau^{-(1+\alpha)} / (\Delta t^{-\alpha} - \tau_c^{-\alpha})$ where Δt is the simulation time step. This distribution has two parameters: the anomalous exponent α and the cut-off time τ_c . When $\tau_c \rightarrow \infty$ or, equivalently $\tau_c > t_{\text{max}}$ (where t_{max} is the maximal simulation time), diffusion is anomalous at all simulation times and equilibrium can never be reached in simulations.

To overcome this issue (and to improve the biological realism of our simulations), we introduced the cut-off τ_c as an upper-limit of the waiting times. In this case diffusion is transiently anomalous (subdiffusive), until a crossover time (that increases with τ_c), after which diffusion gets back to a Brownian regime (see Figure 1B in our article). One major result of our article (2) is that with such CTRW-based transient subdiffusion, the equilibrium fraction of bound receptors appears to vary when we vary α (Figure 2C and 3B3) or τ_c (Figure 2D).

Yet, as rightly pointed out by V.P. Shkilev in his comment, these two parameters define the diffusion coefficient of this transport process according to $D =$

$(\Delta x)^2 [4 \int_{\Delta t}^{\tau_c} \tau \phi(\tau) d\tau]^{-1}$ where Δx is the lattice spacing. Therefore our simulations suggest that, contrarily to Brownian motion, the equilibrium fraction of bound receptors changes with the diffusion coefficient when molecules move via CTRW-based transient subdiffusion.

We moreover found that the equilibrium fraction of bound receptors with CTRW-based transient subdiffusion is actually lower than the Brownian case at least for small diffusion coefficients (see small values of α and large values of τ_c in our Figures 2 and 3). This result disagrees with the mean-field equations obtained by V.P. Shkilev in his comment (1) (eq. 14-19).

We see four major reasons that can explain this discrepancy. First, it has already been realised at several occasions that contrarily to Brownian motion, when CTRW-based subdiffusion is coupled to the reaction terms, the macroscopic behavior of the system might be very sensible to microscopic details of the system, for instance whether reactions can occur during molecule waiting times or only in association with molecule jumps (5, 6). Such an explanation may be at work in our case.

Alternatively, the ‘‘random trap model’’ used by V.P. Shkilev in his comment (and his related article (7)) gives rise to a transient subdiffusive behavior with a very specific non-linear increase of the mean-squared displacement $\langle r^2(t) \rangle = 6D\{t + (b-1)\tau(1 - \exp(-t/\tau))\}$ (eq. 24 in Shkilev (7)). Like the mean-squared displacement observed in CTRW-based transient subdiffusion (our Figure 1B), this formula gives a Brownian (linear) dependence on time for $t \rightarrow 0$ and $t \gg \tau$ and a non-linear behavior in between. However, the general shape of the non-linear regime is quite different from what is obtained with CTRW-based transient subdiffusion, in particular the duration of the non-linear regime can be much larger with CTRW-based transient subdiffusion (several decades as in our Figure 1B) than with the random trap model. In a context where, as already note above, the macroscopic behavior of the system is known to critically depend on microscopic details, such a discrepancy might be important.

Another possible explanation can be found in the ‘‘weak-ergodicity breaking’’ property of CTRW-based subdiffusion, for which time-averages are not equivalent to ensemble averages (8). Even though the subdiffusion regime is transient in our case, the effect of the weak ergodicity breaking could still be significant. In this case, the ensemble averages we use throughout our simulations might not be adequate ways to measure species concentration.

Finally, the mean-field equations developed by V.P. Shkilev in his comment are based on a one-step process for the ligand-binding equilibrium, i.e. eq. (1) above (note that, to

the best of our understanding, his previously published work has not focused on the reversible bimolecular reaction). As such, his mean-field analysis of the Brownian case (eq.17-19 in his comment) fails to predict the fact that the equilibrium fraction of bound receptors in the Brownian case should not depend on the diffusion coefficient. We think that theoretical analysis of the ligand-binding equilibrium as a two step-process including transient subdiffusive transport similar to what was achieved e.g. in Lauffenburger and Linderman (3) for Brownian diffusion (i.e. eq. (2) above), could help resolve the contradiction between our simulation results and the mean-field analysis.

The theoretical and simulation analysis of the coupling between complex reaction schemes and subdiffusion is still in its infancy in spite of the effort of several groups. The results obtained hitherto have already produced several results that are far from trivial compared to their Brownian counterpart (see e.g. (9, 10)). The dependence on diffusion coefficients of fundamental biological processes like the ligand-binding equilibrium might provide another example.

REFERENCES and FOOTNOTES

1. Shkilev, V., 2014. Comments to the Editor. Comment on “Anomalous versus slowed-down Brownian diffusion in the ligand-binding equilibrium”. *Biophysical Journal* .
2. Soula, H., B. Caré, G. Beslon, and H. Berry, 2013. Anomalous versus slowed-down Brownian diffusion in the ligand-binding equilibrium. *Biophysical Journal* 105:2064–2073.
3. Lauffenburger, D. A., and J. J. Linderman, 1993. Receptors: Models for Binding, Trafficking, and Signaling. Oxford University Press, USA.
4. Shoup, D., and A. Szabo, 1982. Role of diffusion in ligand binding to macromolecules and cell-bound receptors. *Biophysical Journal* 40:33 – 39.
5. Hornung, G., B. Berkowitz, and N. Barkai, 2005. Morphogen gradient formation in a complex environment: an anomalous diffusion model. *Phys Rev E* 72:041916.
6. Yuste, S. B., E. Abad, and K. Lindenberg, 2010. Reaction-subdiffusion model of morphogen gradient formation. *Phys Rev E* 82:061123.
7. Shkilev, V., 2005. A model of anomalous transport. *Journal of Experimental and Theoretical Physics* 101:562–567.
8. Barkai, E., Y. Garini, and R. Metzler, 2012. Strange kinetics of single molecules in living cells. *Physics Today* 65:29+.
9. Henry, B. I., T. A. M. Langlands, and S. L. Wearne, 2006. Anomalous diffusion with linear reaction dynamics: from continuous time random walks to fractional reaction-diffusion equations. *Phys Rev E* 74:031116.
10. Fedotov, S., and S. Falconer, 2012. Subdiffusive master equation with space-dependent anomalous exponent and structural instability. *Phys. Rev. E* 85:031132.