

## Comments to the Editor

### Reply to the Comment by V. P. Shkilev on “Anomalous versus Slowed-Down Brownian Diffusion in the Ligand-Binding Equilibrium”

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:

1. When diffusion is uniformly Brownian (with a space-independent diffusion coefficient), “the equilibrium fraction of bound receptors [...] should depend on the diffusion coefficient” and
2. 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.

Whereas 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

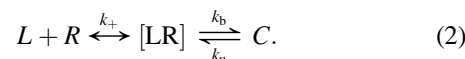
Even when the movement of each molecule is a simple Brownian motion with constant diffusion coefficient, the influence of diffusion on the ligand-binding equilibrium is nontrivial,



where  $k_{\text{on}}$  and  $k_{\text{off}}$  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 Linderman and Lauffenburger (3). We recall below their major results.

The central point is that the classical one-step process depicted in 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, which 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  $k_u$ , which do not depend on the transport process. In agreement with the celebrated Smoluchowski’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  $k_{\text{on}}$  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 Fig. 2 A of our article (2) where the time needed to reach equilibrium increases when the diffusion coefficient (thus  $k_{\text{on}}$ ) decreases.

Now, when applied to the global backward constant rate  $k_{\text{off}}$ , this approach leads to the remarkable conclusion that the global backward step is not a local process; it depends on diffusivity as well. 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 is  $k_{\text{off}} = 0$  in this case, which illustrates the fact that  $k_{\text{off}}$  too depends on the diffusion coefficient.

Theoretical approaches by, e.g., Lauffenburger and Linderman (3) and Shoup and Szabo (4) indeed lead to

$$k_{\text{off}} = \lambda k_u,$$

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

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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. Because the global association rate constant  $k_{\text{on}}$  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.

Contrary 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 Fig. 2 A 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 Fig. 3 B1 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  $\Delta t$  is the simulation time step. This distribution has two parameters: the anomalous exponent  $\alpha$  and the cut-off time  $\tau_c$ . When  $\tau_c \rightarrow \infty$  or, equivalently  $\tau_c > t_{\text{max}}$  (where  $\tau_{\text{max}}$  is the maximal simulation time), diffusion is anomalous at all simulation times and equilibrium can not be reached in simulations.

To overcome this issue (and to improve the biological realism of our simulations), we introduced the cut-off  $\tau_c$  as an upper-limit of the waiting times. In this case, diffusion

is transiently anomalous (subdiffusive), until a crossover time (that increases with  $\tau_c$ ), after which diffusion gets back to a Brownian regime (see Fig. 1 B 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  $\alpha$  (Figs. 2 C and 3 B3) or  $\tau_c$  (Fig. 2 D).

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

$$D = (\Delta x)^2 \left[ 4 \int_{\Delta t}^{\tau_c} \tau \phi(\tau) d\tau \right]^{-1},$$

where  $\Delta x$  is the lattice spacing. Therefore, our simulations suggest that, contrary 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  $\alpha$  and large values of  $\tau_c$  in our Figs. 2 and 3). This result disagrees with the mean field equations obtained by V. P. Shkilev in his Comment (1) (Eqs. 14–19).

We see four major reasons that can explain this discrepancy.

The first possible explanation is that contrary to the case of 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.

A second possible explanation is that 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 nonlinear 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 Fig. 1 B), this formula gives a Brownian (linear) dependence on time for  $t \rightarrow 0$  and  $t \gg \tau$  and a nonlinear behavior in between. However, the general shape of the nonlinear regime is quite different from what is obtained with CTRW-based transient subdiffusion, in particular the duration of the nonlinear regime can be much larger with CTRW-based transient subdiffusion (several decades as in our Fig. 1 B) than with the

random-trap model. In a context where, as already noted above, the macroscopic behavior of the system is known to critically depend on microscopic details, such a discrepancy might be important.

A third 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.

The fourth possible explanation is that 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 (Eqs. 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 Lauffenberger 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 despite 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., Henry et al. (9) and Fedotov and Falconer (10)). The dependence on diffusion coefficients of fundamental biological processes like the ligand-binding equilibrium might provide another example.

Hédi Soula,<sup>†‡\*</sup> Bertrand Caré,<sup>†‡</sup> Guillaume Beslon,<sup>†§</sup> and Hugues Berry<sup>†§\*</sup>

<sup>†</sup>*Institut National de Recherche en Informatique et en Automatique, Villeurbanne, France,*

<sup>‡</sup>*Université de Lyon, Institut National de la Santé et de la Recherche Médicale UMR1060, Villeurbanne, France; and*

<sup>§</sup>*Laboratoire d'Informatique en Image et Systèmes d'Information, Université de Lyon, UMR 5205, Centre National de la Recherche Scientifique-Institut National des Sciences Appliquées, Villeurbanne, France*

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