Comment to the Editor

Comment to the Article by Michael J. Saxton: A Biological Interpretation of Transient Anomalous Subdiffusion. I. Qualitative Model

In a recent article (1), Michael J. Saxton proposes to interpret as anomalous diffusion the occurrence of apparent transient subdiffusive regimes in mean-squared displacement (MSD) plots, calculated from trajectories of molecules diffusing in living cells and acquired by single particle (or molecule) tracking techniques (SPT or SMT). The demonstration relies on the analysis of both three-dimensional diffusion by Platani et al. (2) and two-dimensional diffusion by Murase et al. (3). In particular, the data reported by Murase et al. cover extremely large timescales and experimental conditions: video rate but also high-speed SPT and single fluorescence molecule imaging. This is an exciting opportunity to address the question of anomalous diffusion because the experiments cover timescales ranging from 33 μs up to 5 s, i.e., a range of more than five orders of magnitude(Fig. 1 *b*).

As pointed out by M. J. Saxton, anomalous diffusion (4) arises from an infinite hierarchy of space or energy scales hindering normal diffusion. The normal diffusion law $\mathrm{MSD}(t) = 4D_\mu t$, where D_μ is the microscopic diffusion coefficient, becomes $\mathrm{MSD}(t) \approx \Omega t^\alpha$, where Ω is some coefficient and α is the anomalous diffusion exponent. In the case of subdiffusive behavior, $\alpha < 1$. However, in cellular processes the hierarchy is always finite since there is a short distance cutoff that is larger than the molecular scale, and a large distance one that is typically cell size. Therefore, one can expect an anomalous diffusion regime on a transient time interval only and crossovers to normal diffusion at short and long timescales. It is precisely what Platani et al. (2) and Murase et al. (3) observed. In Fig. 1, the experimental apparent subdiffusive regimes can cover up to three orders of magnitude.

Anomalous diffusion is frequently invoked to interpret complex experimental data. However, the elucidation of the physical mechanisms at its origin remains a difficult and still open issue (5). In this context, the systematic research of the simplest mechanisms accounting for experimental observations should be preferred to avoid an over-interpretation of data. Without questioning the existence of subdiffusive behaviors, which certainly play a key role in numbers of mechanisms in living systems, we would like to point out that the data used by J. M. Saxton can be fitted as well by a simple law, resulting from confined diffusion at short times, with a slower free diffusion superimposed at larger times:

$$\mathrm{MSD}(t) = L^2(1-\exp(-t/\tau))/3 + 4D_\mathrm{M}t, \qquad (1)$$

where there is now only one length-scale, L, the typical size of the confining domains. The timescale $\tau = L^2/(12\ D_\mu)$ is the equilibration time in the domains (8). D_M is the long-term diffusion coefficient, ensuing, for example, from the fact that the confining domains are semi-permeable (6). This law is a very good approximation of a more complex form (7) because it takes into account only the slowest relaxation mode of confined diffusion at short times (8). By contrast, the contribution of the free long-term diffusion is mathematically exact. It can be proven (calculations not shown) that this contribution is equal to $L^2/3 + 4\ D_\mathrm{M}\ t$, consistent with Eq. 1. In addition, the short-term expansion of Eq. 1 gives $\mathrm{MSD}(t) = 4(D_\mu + D_\mathrm{M})t$ when $t \ll \tau$, where one would expect $\mathrm{MSD}(t) = 4D_\mu t$. This is because the calculation we referred to above does not take into account the correct time distribution of domain-to-domain jumps when $t \leq \tau$. It overestimates the probability of jumps at very short times. This problem, that will be addressed elsewhere, is beyond the scope of this Comment. Indeed, we work in the regime $D_\mu \gg D_\mathrm{M}$, where this issue is negligible, as confirmed in the simulations below. Fig. 1 illustrates that this law accounts quite well for the observed transient regimes without appealing for anomalous diffusion. Within this approximation (Fig. 1 *b*), the fit of experimental data by Eq. 1 gives $D_\mu = 0.36\ \mu\mathrm{m}^2/\mathrm{s} = 10D_\mathrm{M}$. The numerical values that we get are consistently close to those of Murase et al. (3). In Fig. 1 *a*, the MSD is calculated from three-dimensional positions (2), and Eq. 1 must be multiplied by 3/2 to be adapted to three dimensions. In both sets of data (Fig. 1, *a* and *b*), the apparent anomalous exponents measured by M. J. Saxton are the slopes of the MSD/*t* profiles at their inflection points, in log-log coordinates.

To confirm further our statements, we have performed numerical experiments of Brownian particles diffusing in a mesh-grid of semipermeable linear obstacles. The complete simulation procedure was detailed in Meilhac et al. (6). Our results are summarized in Fig. 2 where numerical MSD(*t*)/*t* plots are fitted by Eq. 1. Two conclusions can be drawn: 1), as anticipated, Eq. 1 is a very good approximation of the real diffusive properties of the system considered; and 2), between the short- and long-term regions where MSD is proportional to *t*, there is an intermediate region, the duration of which is comparable to the ratio D_μ/D_M (in logarithmic scale). In this region, the MSD/*t* plots resemble anomalous diffusion plots, with slope tending to -1 when the previous ratio is large. Indeed, when $D_\mu \gg D_\mathrm{M}$, the log-log plot of

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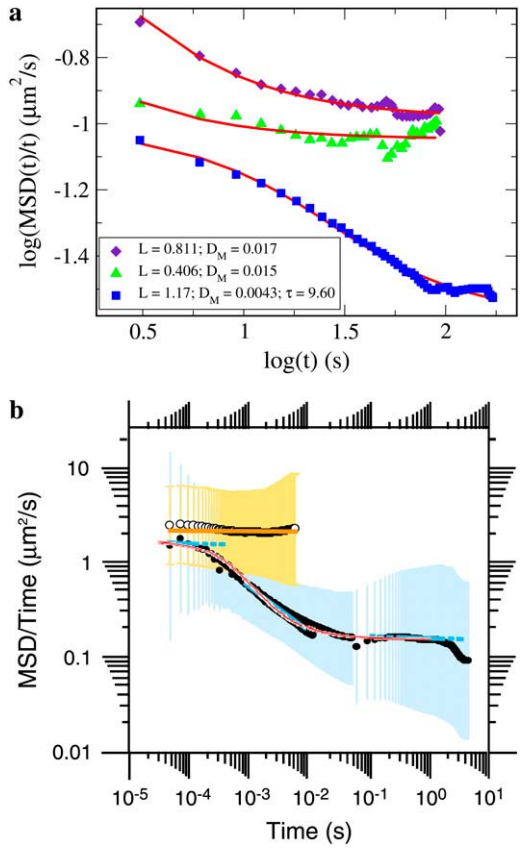

FIGURE 1 Log-log plots of experimental mean-square displacements divided by time (MSD/t) versus time t, where normal diffusive regimes are characterized by a constant value whereas apparent subdiffusivity is revealed by quasi-linear regimes with negative slopes: (*a*) For Cajal bodies (adapted from Platani et al. (2)); experimental data (*symbols*) are suitably fitted by Eq. 1 (*lines*). The fit parameters L (in μm) and D_M (in $\mu m^2/s$) are given in the inset. The microscopic diffusion regime (i.e., τ (in s) or equivalently D_μ) is accessible only for the lowest set of data (*squares*), because τ is too small for the two remaining sets. (*b*) For dioleoylPE (*solid circles*, with error bars, adapted from Murase et al. (3)), power law fits in both the normal and anormal apparent diffusive regimes (*blue*, (1)). In red, our best fitting curve for time < 1 s, with $L = 35$ nm, $\tau = 0.28$ ms, and $D_M = 0.036\ \mu m^2/s$ (see Eq. 1).

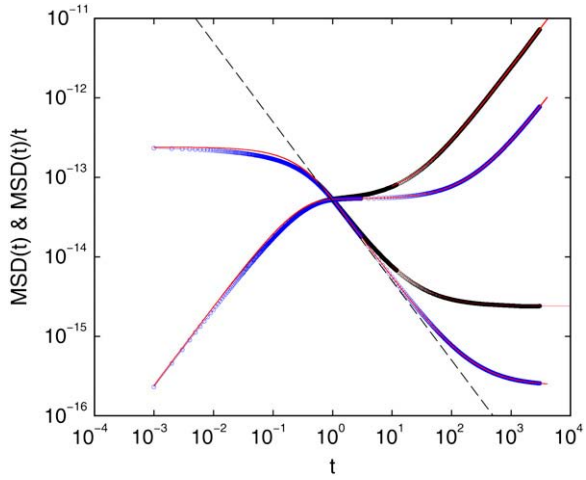

FIGURE 2 Numerically simulated (*symbols*; L = 400 nm, $\tau = 0.22$ s, $D_\mu = 0.06\ \mu m^2/s$) MSD(t) (*lower plots on the left-hand-side*) and MSD(t)/t (*upper plots*), for $D_\mu/D_M = 10^2$ (*black*) and 10^3 (*blue*), on which are superimposed the MSD(t) and MSD(t)/t calculated from Eq. 1 (*lines*). MSD(t)/t for $D_\mu/D_M = 10$ was given in Fig. 1 *b*. The agreement is excellent except around τ where Eq. 1 is only an approximation (see text). The dashed line has slope -1.

MSD(t) is as follows. When $t \ll \tau$, MSD(t) $= 4\ D_\mu t$ and MSD/t is constant. When $t \gg L^2/(12D_M) = \tau_{esc}$, MSD($t$) $= 4\ D_M t$ and MSD/t is also constant. The time $\tau_{esc} = \tau\ D_\mu/D_M$ corresponds to the typical time needed to escape boxes (6). In the intermediate region, MSD(t) $= L^2/3$ is constant. There are two crossovers near τ and τ_{esc}. For the MSD/t representation, the constant transient regime becomes affine with slope -1. When the ratio D_μ/D_M is large but finite, the slope of this intermediate region is still negative, but it is larger than -1. The graph resembles an anomalous diffusion graph on the time interval $[\tau,\tau_{esc}]$ (see also Fig. 1). Note that, up to translations, the shapes of the MSD and MSD/t curves in log-log coordinates only depend on the ratio $\tau_{esc}/\tau = D_\mu/D_M$.

When visualizing MSD plots, the transition from short-term diffusion that is confined in domains of size L to slower, longer-term free diffusion can be confused with anomalous diffusion over several orders of magnitude of time. With the goal of researching the simplest mechanisms accounting for experimental observations, it seems reasonable to explore first the former possibility. In principle, elucidating the nature of domains with a single typical size L is a much easier task than identifying a hierarchy of space (or energy) scales ranging over several orders of magnitude. In the work of Murase et al. (3), domains of size $L \approx 30$ nm are attributed to the cortical cytoskeleton meshwork. In the case of Cajal bodies (2), the fitted values $L \approx 1\ \mu$m will have to be interpreted in future work: the confining roles of chromatin-associated states and of possible division of the nucleus in functionally distinct compartments (2) will have to be investigated.

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