Comment to the Editor

Response to Comment by Destainville et al.

Identifying potential sources of artifactual anomalous diffusion is an important contribution, particularly in the case of transient anomalous subdiffusion. Martin et al. (1), for example, showed that noise in single-particle tracking (SPT) measurements can lead to a period of spurious anomalous subdiffusion. This work originated from experimental evidence of anomalous subdiffusion in a system for which diffusion ought to have been purely normal. In their Comment, Destainville et al. (2) point out that a period of spurious anomalous diffusion can result from the transition between two limiting cases, normal diffusion within a corral (or a cage in three dimensions) at short times, and normal hop diffusion among corrals at long times. (For a review of anomalous diffusion see Metzler and Klafter (3) and for a discussion in a biological context see Condamin et al. (4).)

PARAMETER TUNING

How can true transient anomalous subdiffusion be identified in modeling? In some cases one can conclude that transient anomalous subdiffusion is real from the behavior of the model as a parameter is tuned. For example, for obstructed diffusion on a lattice, there is an initial period of anomalous subdiffusion and a crossover to normal diffusion at long times (5). As shown in Fig. 1, as the obstacle concentration is increased, diffusion becomes more anomalous over longer times. At the percolation threshold, diffusion becomes anomalous at all times, a well-known result, and the anomalous diffusion exponent becomes equal to its known value for diffusion on the percolation cluster.

In the case of a finite hierarchy of traps, the parameter to be tuned is the number of layers in the hierarchy (6). For even a single trap, there is necessarily an inflection point in the plot of $\log\langle r^2\rangle/t$ versus $\log t$, and the linear region around the inflection point is best interpreted as an artifactual period of anomalous subdiffusion (see Fig. 5 of Saxton (6)). But Fig. 2 shows that as the hierarchy is built up, diffusion becomes more anomalous over longer times. Here the limit of an infinite trap hierarchy is similar to the well-known continuous-time random walk (CTRW) model, which gives anomalous subdiffusion at all times. In both the CTRW and the trap hierarchy models, the escape times are given by a power-law distribution. The difference is that in a CTRW, the trap at the occupied site is newly generated from a random distribution at each move (dynamic or annealed disorder), but in the trap hierarchy model, the traps are permanent and immobile (static or quenched disorder). In the CTRW the distribution is continuous; in the trap hierarchy model it is discrete, although this is not essential to the model.

Parameter tuning can be done experimentally as well. In measurements of diffusion of a colloidal probe in an actin gel, Wong et al. (7) tuned from normal to anomalous to elastic regimes by increasing the ratio of the probe size to the average mesh size in the gel.

THE EXPERIMENTAL RESULTS

Consider the experimental curves (Fig. 1 of Destainville et al. (2)) showing apparent transient anomalous subdiffusion. On physical grounds a corral model is plausible in both cases, so the analysis proposed by Destainville et al. (2) may be applicable. Two-dimensional diffusion in the plasma membrane is likely to be obstructed by cytoskeletal elements, as proposed in the corral models of Sheetz (8) and Kusumi et al. (9). Likewise three-dimensional diffusion in the nucleus may be obstructed by chromatin. In both cases, however, binding is also plausible. Proteins permanently or transiently bound to the cytoskeleton form the pickets in the Kusumi picket fence model (9), and binding of certain proteins to sites on chromatin is essential to the function of the nucleus.

DISTINGUISHING THE POSSIBILITIES

How can one distinguish true transient anomalous subdiffusion from artifactual subdiffusion? Several approaches are possible.

1. Modeling. One approach would be to construct a model of corrals including the dynamics of corral walls and diffusion. The model would express the escape time in terms of the probabilities of gate-opening events of various widths and durations, the probability that the diffusing particle will reach an open gate, and the probability that the particle will exit through the gate. The key questions would be, does the distribution of escape times imply anomalous, transient anomalous, or normal diffusion, and does diffusion become more anomalous as one tunes a parameter such as the stiffness of the corral wall or the density of crosslinks?
2. SPT measurements of $\langle r^2(t)\rangle$. The equation proposed by Destainville et al. (2) might be able to distinguish the mechanisms. The curves of Figs. 1 and 2 are not well fit by (segments of) that equation, but a conclusive test would require Monte Carlo results for the continuum, not

Submitted May 19, 2008, and accepted for publication June 24, 2008.

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Editor: Edward H. Egelman.

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0006-3495/08/10/3120/03 $2.00

doi: 10.1529/biophysj.108.136861

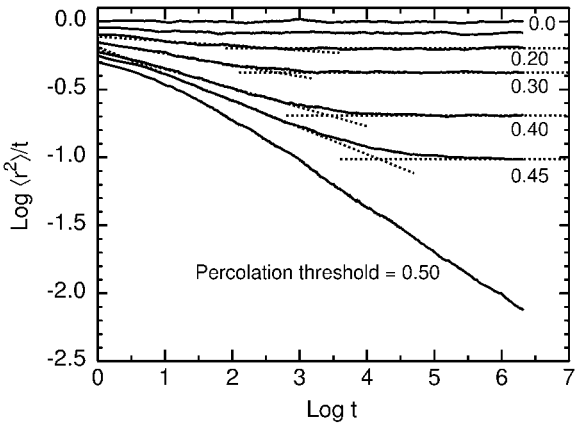

FIGURE 1 Anomalous subdiffusion of a particle on an obstructed lattice as the obstacle concentration approaches the percolation threshold. Monte Carlo results are plotted as $\log\langle r^2\rangle/t$ versus $\log t$, where $\langle r^2\rangle$ is the mean-square displacement and t is time. Anomalous subdiffusion, $\langle r^2\rangle \propto t^{\alpha}$, yields a straight line of slope $\alpha - 1$, where α is the anomalous subdiffusion exponent. Normal diffusion, $\langle r^2\rangle \propto t$, yields a horizontal line. The intersection of these lines defines the crossover time. Obstacle concentrations are 0.0, 0.1, 0.2, 0.3, 0.4, 0.45, and 0.5. The percolation threshold for the triangular lattice is 0.5. Adapted from Saxton (5).

a lattice, because a lattice model integrates out behavior over distances less than the lattice constant. The curves in Figs. 1 and 2 start in the anomalous region because some diffusing particles are initially in contact with obstacles or at a binding site.

3. Refined SPT measurements. The most direct experimental approach would be SPT measurements at high enough resolution to detect motion within the corrals in order to distinguish binding from corralling. Measuring histograms of escape times is essential. Simultaneous measurements of the position of the corral walls is highly useful. The data analysis must distinguish trapping or confinement from the apparent localization that occurs by chance in a pure random walk (10–12).

   Motion within corrals and jumps between them have been observed by SPT in the plasma membrane (9). Similar observations were made for colloidal particles in actin gels (7). The observed anomalous subdiffusion in actin gels was attributed to large rare jumps between cages; the escape time from a cage had a power-law distribution over ~2 1/2 orders of magnitude. Andrews et al. (13) reported caging in their careful SPT measurements on the high-affinity IgE receptor with simultaneous imaging of the actin cortex. SPT measurements of Cajal bodies and chromatin in the nucleus were interpreted in terms of transient binding by Platani et al. (14) though related measurements by Görisch et al. (15) were taken to indicate caging.

4. Inhibitors. In the finite trap hierarchy model, anomalous subdiffusion occurs only for a nonequilibrium initial state (6). In principle, one could use metabolic energy inhibitors to test for this mechanism. However, in cells this test will not distinguish binding from corralling if the actin or chromatin corral walls are constantly remodeled by processes requiring metabolic energy. Inhibitors affecting the stiffness of the corral walls would still be useful.

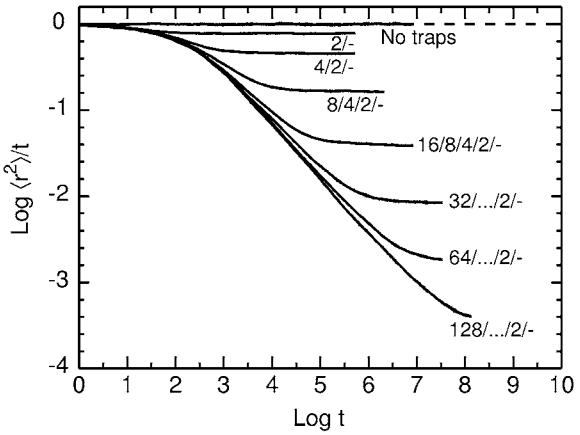

FIGURE 2 Anomalous subdiffusion of a particle in the trap hierarchy model as layers are added to the hierarchy. For example, 8/4/2/- indicates a system containing 8 traps with escape probability p, 4 traps with escape probability p^2, 2 traps with escape probability p^3, and no targets. Monte Carlo results are plotted as in Fig. 1. The system size is varied between 8×8 and 94×94 to keep the total trap concentration as constant as possible, 0.02958 ± 0.00096. The escape probability per time step is $p = 0.1$. Adapted from Saxton (6).

According to one formulation of Occam's razor, ''Entities are not to be multiplied without necessity''. But given the known structural components of cells and their known or plausible interactions, diffusion in a cell involves obstruction, binding, and hydrodynamic interactions with obstacles, all in a crowded system. One must be cautious in invoking Occam's razor to constrain cellular mechanisms when nature has already multiplied the entities, presumably for various biological necessities.

This work was supported by National Institutes of Health grant GM038133.

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