Bursts of Active Transport in Living Cells

Bo Wang,1 James Kuo,2 and Steve Granick1,2,3,4,*

1Department of Materials Science, University of Illinois, Urbana, Illinois 61801, USA
2Department of Chemical and Biomolecular Engineering, University of Illinois, Urbana, Illinois 61801, USA
3Department of Chemistry, University of Illinois, Urbana, Illinois 61801, USA
4Department of Physics, University of Illinois, Urbana, Illinois 61801, USA

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We show, using a large new data set, that the temporally resolved speed of active cargo transport in living cells follows a scaling law over several decades of time and length. The statistical regularities display a time-averaged shape that we interpret to reflect stress buildup, followed by rapid release. The scaling power law agrees quantitatively with those reported in inanimate systems (jammed colloids and granular media, and magnetic Barkhausen noise), suggesting a common origin in pushing through a crowded environment in a weak force regime. The implied regulation of the speed of active cellular transport due to environmental obstruction results in bursts of speed and acceleration. These findings extend the classical notion of molecular crowding.

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One of the most fundamental differences between the inanimate and living world is the prominence of active transport in living systems. This active motion is driven by the proteins known as molecular motors [1,2], and underpins essential functions, including locomotion, cell cycle, signaling, and metabolism. Much is known about the molecular mechanisms of elementary steps taken by the motors [3–6], but not enough about how these steps coordinate in the context of a crowded cellular environment. Here, analyzing a uniquely large data set of individual trajectories of cargo transport in living cells, we reveal statistical commonality with driven motion in jammed nonliving systems and a conceptual connection to delocalization in glassy or jammed dynamics [7–13].

What is known already about active transport is that, when a single motor protein (say, kinesin or dynein) drags a vesicular cargo, such as endosome or lysosome, along a microtubule, the motor is subject to an opposing load force arising from viscous drag [Fig. 1(a)]. Extensive exploration in vitro shows that the speed decreases approximately linearly with respect to increase of the load force and that multiple motors can bind to the cargo simultaneously with load-dependent dissociation rates [5,6]. Then, if the environment just presents viscous drag in existing theoretical models, the cargo speed is expected to take discrete values according to the number of molecular motors, with some noise around these fixed points [Fig. 1(b)] [5]. Here we consider the complexity of cargo that must be dragged through the crowded, heterogeneous, and likely viscoelastic cytoplasm of a living cell [Fig. 1(c)]. We hypothesize that speed would decrease as stress builds up, the environment pushing against cargo motion; then, bursting free of this transient confinement, cargo would speed up. Thus, while it is known that on short time and length scales the speed is dictated by the molecular kinetics [5,6] (∼1 ms, ∼10 nm), slow environmental structural rearrangement might contribute significantly on larger scales. Therefore, the slow fluctuation of the speed is expected to be instructive, but earlier studies in living cells focused instead on the average speed.

To follow active transport in living cells at the single-cargo level, we used fluorescence imaging. Living mammalian cells were imaged under physiological conditions in a homebuilt microscope using highly laminated illumination (HILO) [14] to improve the signal-to-noise ratio and minimize possible errors introduced by 2D projection. To fluorescently label specific populations of endosome cargo, either 0.15 μg/mL biotinylated epidermal growth factor (EGF) complexed to Alexa-555 streptavidin or 10 μg/mL Di conjugated low-density lipoprotein (LDL) (Invitrogen) was applied to cells. To fluorescently label lysosome cargo, cells were transiently transfected with lysosomal-associated membrane protein 1 (LAMP1) fused to tagRFP (Invitrogen). Fluorescence images were taken on the basal side of cells at a frame rate of 50 ms, this time being selected as longer than the motor stepping time (1–10 ms) to remove molecular noise. The center positions of the cargos were each located with a resolution of <5 nm and strung together by an automated program to form trajectories [15].

The active transport portions of trajectories were identified by wavelet analysis [16] and the speed along the (sometimes-curved) active transport path was calculated over 50 ms time intervals. To confirm self-consistency of the data, we inspected displacements perpendicular to the track, and found them to be Gaussian in distribution with variance of 20 nm; this reasonable number coincides with the sum of microtubule radius and length of motor stalk, indicating perpendicular displacements are mainly contributed from transverse swinging of cargos on our time scale. Furthermore, when 5 μg/mL...
nocodazole was supplemented in the medium to disassemble microtubules, no active transport was observed. This confirmed that the active transport reported below was along microtubules.

Raw data of position, velocity, and acceleration are illustrated in Fig. 2. We tracked a large number of cells, >50 cells for each experimental condition, and the patterns of data in this figure were verified for >10,000 runs of uninterrupted unidirectional motion under each condition. Panel (a) shows a typical run with color denoting elapsed time, one notices that motion is sometimes slow, sometimes fast. Panel (b) shows two examples of temporally resolved speed versus time; one notices asymmetric bursting dynamics, different from ordinary noise around a mean value. Panel (c) plots speed against acceleration over a large range of variables (left plot) and with a magnified view of the same data (right plot). The spiral patterns show that speed fluctuations have a nonlinear dynamic feature with memory as long as one second, a time that coincides with the known typical mechanical relaxation time of cells [13]. Limit cycles are not observed, so this motion is not periodic, but the circuits are parallel and tilted. In other words, while fluctuations differ in both amplitude and time, their shapes are similar and they are regularly asymmetrical with time.

Speed fluctuations do not appear to cluster around distinct values, nor do they present distinct peaks of multiple Gaussian distributions, features that would be expected if they reflect motor association-dissociation events [5,17].

Next, we considered correlations. Ensemble-averaged speed-speed autocorrelation functions of active cargo transport are plotted in Fig. 3 against time. The negative correlation peak is pronounced at ~0.3 s, yet negative over a significantly broader time, confirming the long memory already noted. Physically, we interpret this to indicate a
broad distribution of the time scales of fluctuations, a ubiquitous feature of dynamic heterogeneity. This is in contrast to a sharp peak. A sharp peak (not observed) would correspond to a well-defined time scale, which would be anticipated if the observed nonlinearity were induced by a specific molecular biochemical cycle.

For further quantification, we analyzed the duration and travel distance of each fluctuation cycle. Given a time series of speed \( v(t) \) for a single run, we imposed a reference \( \tilde{v} \) defined as 60% of the most likely speed in this run, and defined “bursts” as periods of time when speed exceeded this reference. Each burst has a duration \( T \) measured as the time between two successive intersections of \( v(t) \) with \( \tilde{v} \). Each burst also has a distance traveled within this run, the burst length \( L \). Figure 4(a) (top) shows extremely broad distributions of burst length as anticipated for classic dynamic heterogeneity. However, when the rather noisy spectrum of raw data is averaged, the striking phenomenological power law is observed over more than 2 decades of \( T \), \( L \sim T^{3/2} \). It is not known whether the slight bending down at longest \( T \) might reflect limited statistics. In addition to these HeLa cells we confirmed the pattern in another mammalian cell type, Marc-145. This power of \( 3/2 \), independent of how the reference speed \( \tilde{v} \) is defined, matches phenomenologically that expected from crackling noise in critical inanimate systems \[7,18\]. Importantly, disrupting actin filaments with latrunculin A (LatA) or cytochalasin D (CytoD) switches to a scaling power of bursts less than \( L = 100 \text{ nm} \) and \( T = 0.3 \text{ s} \) after disrupting actin networks with CytoD. This change is more evident in the inset magnifying the regime indicated by the dashed box. Symbol representations are the same as Fig. 3.

FIG. 3 (color online). The ensemble-averaged speed-speed autocorrelation function \( R(\Delta t) \) of active transport. (a) The shape of \( R(\Delta t) \) vs \( \Delta t \) is invariant to cargo types that are known to be transported by different sets of scaffold and motor proteins through separate pathways, indicating molecular details only play a minor role. (b) In contrast, a systematic change is observed when actin was disrupted. The curves are from EGF-containing endosomes in cells treated with 0.75 \( \mu \text{g/mL} \) CytoD or 0.08 \( \mu \text{g/mL} \) LatA. The LatA and CytoD concentrations were chosen such that blebs formed at cell peripheries but cells retained their shapes and no complete rounding was observed within 3 h. The solid line in (b) depicts the EGF-containing endosomes in actin-intact cells. The dashed lines are single exponential fits for short time decay. To compute the autocorrelation consistently, we cropped runs longer than 50 frames into pieces of length 50 frames or shorter.

FIG. 4 (color online). Scaling of speed fluctuations for the active transport of endosomes. (a) Log-log plot of burst length \( L \) versus their duration \( T \); these data refer to EGF-containing endosomes. Averaged from 26 496 bursts, the crosses (raw data shown as gray circles), have the slope indicated in the figure. (b) Collapse of such data by normalizing the length with \( L_0 \) such that \( L/L_0 = 1 \) when \( T = 1 \text{ s} \). The solid lines have the slopes indicated in the figure, highlighting a change of the power law when \( L < 100 \text{ nm} \) and \( T < 0.3 \text{ s} \) after disrupting actin networks with CytoD. This change is more evident in the inset magnifying the regime indicated by the dashed box. Symbol representations are the same as Fig. 3.
CytoD, granular media, and magnetic Barkhausen noise \cite{7,18}, reported in inanimate systems (jammed colloids and observed here is in quantitative agreement with those properties. The universality of the scale-free power law scaling transport in living cells shows striking statistical regular-

motor proteins. This was insensitive to changing the molecular details of the mechanics that has been proposed by others \cite{12,13}. All active transport, which in turn relates to universality in cell complex regulatory pathways and genes, and it is also true at the molecular level this endogenous transport surely involves simple heuristic argument that these data reflect the superposition of ballistic motion and random Brownian noise \cite{19}. Taken together, although it is true that at the weak force regime.

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*To whom correspondence should be addressed. sgranick@uiuc.edu

\begin{figure}[h]
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\includegraphics[width=\columnwidth]{figure5.png}
\caption{(color online). 2D probability distributions of burst duration and length of EGF-containing endosomes in untreated and actin disrupted cells as identified in the 3 panels. The bins in length and time are 50 nm and 50 ms, respectively. The color is coded as the logarithmic probability covering 2 orders of magnitude with red showing high probability and blue showing low probability.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\columnwidth]{figure6.png}
\caption{(color online). The average burst shape, obtained by rescaling all bursts longer than 0.3 s to the same duration (T). For averaging, only the time periods above the reference \( \dot{v} \) were included, and the reference level was subtracted from the speed. The curves of different conditions were normalized by the area under the curve; thus, the unit of the \( y \) axis is arbitrary. Note that curves are smooth as the noise in individual bursts averages out. Compared to the solid line, a semicircle, the asymmetry of the data is evident. For comparison to the linear plot (left), the tail for small fractional period, \( t/T \), is plotted on a log-log scale (right). The dashed line through the data has slope of 1 indicating an accelerating stage. In comparison, the tail of the semicircle (solid line) has slope close to 1/2. Importantly, the data under all conditions collapse. Symbol representations are the same as Fig. 3.}
\end{figure}

observe differences only for \( L \) smaller than this. Consistently, after actin disruption, the first minimum in speed-speed autocorrelation curves becomes shallower, indicating a shift towards longer times [Fig. 3(b)]. Likewise, higher probability of larger bursts was observed after actin disruption, as one sees in a plot of the 2D probability density of burst length and duration in Fig. 5. Taken together, these observations indicate that disruption of the actin network loosens the constraint on cargo transport.

To better characterize the burst shape, we rescaled time \( t \) for each individual burst by burst-specific duration \( T \) and speed by \( T^{1/2} \). This representation (Fig. 6) shows that the average burst is asymmetric in time. This is consistent with the picture that as cargo moves, dragged by molecular motors, at first stress builds up such that motion slows, then the stress releases and motion speeds up (Fig. 6). The initial acceleration during which speed increases linearly with time (the magnified view in Fig. 6 right panel) may reflect release of elastic stress. The power law with slope 3/2 between burst length and duration is consistent with the simple heuristic argument that these data reflect the superposition of ballistic motion and random Brownian noise \cite{19}. Taken together, although it is true that at the molecular level this endogenous transport surely involves complex regulatory pathways and genes, and it is also true that the burst shape should depend on the detailed relaxation mechanism of the environment, the power laws demonstrated here suggest scale-free simplicity, when coarse grained by the normalizations we have made. This invariance highlights the redundancy in regulation of cellular active transport, which in turn relates to universality in cell mechanics that has been proposed by others \cite{12,13}. All this was insensitive to changing the molecular details of the motor proteins.

In summary, the temporally resolved speed of active transport in living cells shows striking statistical regularities. The universality of the scale-free power law scaling observed here is in quantitative agreement with those reported in inanimate systems (jammed colloids and granular media, and magnetic Barkhausen noise \cite{7,18}), suggesting a common origin in pushing through a crowded environment in the weak force regime.

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