

Anisotropic Subdiffusion in the Cytoplasm of Living Cells

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The cytoplasm of eukaryotic cells is a complex fluid that harbors a variety of membrane-enveloped organelles with distinct spatial distributions and duties. Peroxisomes, for example, are vesicular entities with radii in the sub-micron range, dispersed all over the cytoplasm to provide oxidative reaction vessels across the cell. Here, we have tracked peroxisomes as biologically relevant tracer particles to explore the local structure of the cytoplasm. Focusing on the vast majority of (sub)diffusively moving peroxisomes, we show that these display a significant motion anisotropy even in the absence of intact actin filaments and microtubules or when the endoplasmic reticulum (ER) is disrupted. In untreated cells, the local preference direction aligns with a global axis that correlates with the cell's long axis; disrupting the cytoskeleton or the ER progressively randomizes this alignment. Altogether, our data indicate that the cytoplasm features a weak nematic substructure rather than being an isotropic fluid.

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The interior of eukaryotic cells is tidily organized into compartments (“organelles”) that provide distinct chemical milieus for specific duties. Peroxisomes, for example, are membrane-enveloped organelles with diameters 0.1–1 μm that host an oxidative environment in which, for example, long-chain fatty acids, are catabolized [1]. Peroxisomes are also key for regulating cellular hydrogen peroxide levels (hence their name), protecting the cell from uncontrolled and harmful oxidation events.

In mammalian cells, a few tens to hundreds of peroxisomes are dispersed over the cytoplasm [cf. Fig. 1(a) for an example], most likely to support their role as omnipresent protective scavengers that eliminate toxic metabolic products. A dispersed state may also facilitate an equal partitioning of peroxisomes during cell division [2]. Key to this spatial arrangement are (transient) associations of peroxisomes with microtubules via suitable molecular motors that mediate long-range transport between the cell center and the periphery (see Ref. [2] for a brief review). A recent study has shown, however, that only a few percent of all peroxisomes show a ballistic motion along microtubules for more than five consecutive steps [3], whereas the vast majority instead shows local and erratic motion in the cytoplasm.

Based on these observations, we hypothesized that peroxisomes could serve as prototypical test particles that report on local features of the cytoplasm as seen by a biologically relevant probe. Earlier work has shown that artificial tracer particles, which have been forced into cells, interact unspecifically with cytoplasmic structures, hence exhibiting a (partly intermittent) anomalous diffusion

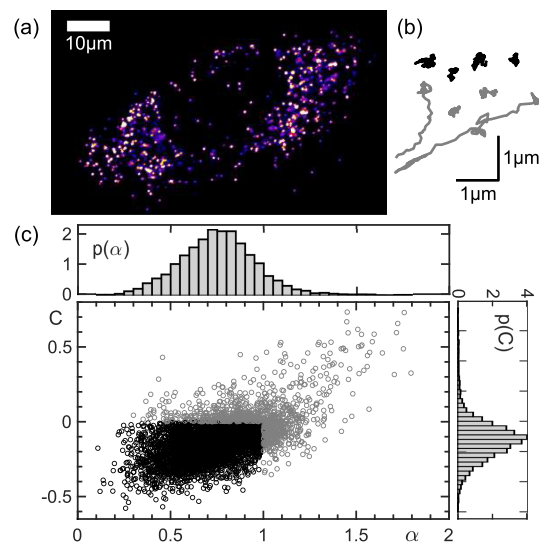


FIG. 1. (a) Confocal fluorescence image of a U2OS cell with peroxisomes highlighted by SKL-GFP (Video in Supplemental Material [4]). (b) Example trajectories of peroxisomes that were classified as (sub)diffusive and superdiffusive (black and gray, respectively). (c) Trajectories were classified as superdiffusive (gray) versus (sub)diffusive (black) when their TA-MSD scaling exponent was $\alpha > 1$ or when the VACF value was $C > 0$.

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characteristic [6–8]. Unlike artificial beads, peroxisomes are native probes that interact in a biologically regulated fashion with molecular motors, cytoskeletal filaments, and extended endomembrane structures like the endoplasmic reticulum (ER) [9] or mitochondria [10]. Therefore, one may expect that they can reveal biologically relevant features of the cytoplasm that remain hidden when monitoring artificial tracer particles.

Following this rationale, we have performed extensive single-particle tracking experiments on peroxisomes in living U2OS culture cells with a time resolution of $\Delta t = 110$ ms between successive frames (see Materials and Methods [4]). The in-plane motion of individual peroxisomes could be tracked over hundreds of frames [see Fig. 1(b) for examples], and trajectories with at least $N = 100$ consecutive positions (typically a few thousands per cell) were used for subsequent analyses. In line with our previous approaches [8,11], we first calculated for each trajectory the time-averaged mean square displacement (TA-MSD), $\langle r^2(\tau) \rangle_t = \langle [\mathbf{r}(t + \tau) - \mathbf{r}(t)]^2 \rangle_t$, and their ensemble average (EA-TA-MSD) in a cell. All MSDs were fitted with a simple power law $\langle r^2(\tau) \rangle = 4K\tau^\alpha$ in the range $\tau \leq 5$ s [4]. Here, the scaling exponent α indicates (sub)diffusive ($\alpha \leq 1$) or superdiffusive ($\alpha > 1$) motion, with K denoting the generalized diffusion coefficient. In addition, we calculated for each trajectory the velocity autocorrelation function (VACF), $\mathcal{C}(\tau) = \langle \mathbf{v}(t) \cdot \mathbf{v}(t + \tau) \rangle_t / \langle \mathbf{v}(t)^2 \rangle_t$ with $\mathbf{v}(t) = [\mathbf{r}(t + \delta t) - \mathbf{r}(t)] / \delta t$. The characteristic value $C = \mathcal{C}(\tau = \delta t)$ is an indicator for the type of motion since (sub)diffusion typically yields $C \leq 0$, whereas superdiffusive motion can be expected to yield $C > 0$ [11,12]. While we focus below on $\delta t = \Delta t$, we confirmed that choosing $\delta t / \Delta t = 1, \dots, 9$ did not lead to significant changes in the results.

In agreement with previous observations, we found that peroxisomes mostly show a (sub)diffusive motion, i.e., only 15%–20% of all trajectories featured a superdiffusive signature [$\alpha > 1$ or $C > 0$; see example in Fig. 1(c)]. Dissecting trajectories into complementary subensembles of (sub)diffusive ($\alpha \leq 1$ and $C \leq 0$) and superdiffusive trajectories, the associated EA-TA-MSDs showed scaling exponents $\alpha \approx 0.7$ (similar to colloidal probes [7,8]) and $\alpha \approx 1.1$, respectively. Because of the small superdiffusive pool, even the EA-TA-MSD without dissection showed a clear sublinear scaling [Fig. S1(a) in Supplemental Material [4]].

Long-range transport of peroxisomes has been shown to depend on cytoskeletal motors [13]. Therefore, the small superdiffusive pool of trajectories can be rationalized to emerge by a simple stochastic switching between local diffusive motion and ballistic transport along cytoskeletal filaments. In contrast, the larger ensemble of (sub)diffusive trajectories, to which we will restrict our analysis in the remainder, can be expected not to include significant contributions of motor-driven transport along cytoskeletal elements.

In line with this notion, (sub)diffusive trajectories were seen to feature increments between successive frames,

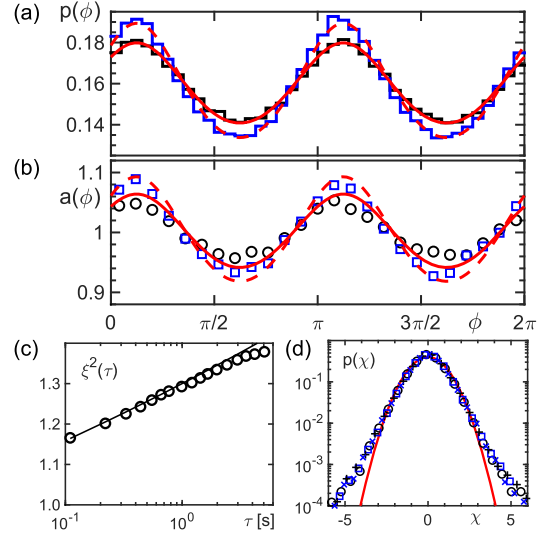


FIG. 2. (a) The angular PDF $p(\phi)$ of steps taken by peroxisomes in the cell shown in Fig. 1(a) within one or ten frames (black and blue lines, respectively) features a marked modulation around the trivial value $1/(2\pi)$ with two distinct maxima of equal magnitude at $\phi = \phi_0, \phi_0 + \pi$ ($\phi_0 \approx \pi/8$). This clear anisotropy of step directions in (sub)diffusive trajectories is well captured by Eq. (1) with $\xi = 1.13$ and $\xi = 1.19$ (full and dashed red lines), respectively. (b) Alongside, the relative step variation $a(\phi)$, defined in the main text, shows a similar modulation that is well captured by Eq. (2) without any additional parameters. (c) The squared step size anisotropy, $\xi^2(\tau)$, defined as the ratio of EA-TA-MSDs $\parallel \mathbf{e}_{\phi_0}$ and $\perp \mathbf{e}_{\phi_0}$, grows with the period τ in which the steps are taken [symbols; full line is an empirical fit $\xi^2(\tau) = 1.3\tau^{0.05} \mu\text{m}^2/\text{s}^{0.05}$]. (d) The PDF of normalized step increments, $p(\chi)$, is the same for steps $\parallel \mathbf{e}_{\phi_0}$ and $\perp \mathbf{e}_{\phi_0}$ (black and blue symbols, respectively). Also the period in which the step was taken (crosses: $\tau = \Delta t$; open symbols: $\tau = 10\Delta t$) did not alter $p(\chi)$. In all cases, marked deviations from a normal distribution (red line) are seen, indicating a considerable heterogeneity of the (sub)diffusive motion within each trajectory.

$\Delta \mathbf{r} = \mathbf{r}(t + \Delta t) - \mathbf{r}(t)$, that appear consistent with an isotropic motion [see Fig. S1(b) in [4] for an example]. However, transforming increments to polar coordinates, $\Delta \mathbf{r} \mapsto (\Delta r_\phi, \phi)$, and inspecting the angular probability distribution function (PDF), $p(\phi)$, regardless of the associated step length Δr_ϕ , a clear modulation with two distinct maxima of equal magnitude at $\phi = \phi_0$ and $\phi = \phi_0 + \pi$ was seen in all cases [see example in Fig. 2(a)]. This modulation indicates that increments of the (sub)diffusive peroxisome trajectories have a directional preference with a nematic symmetry, i.e., bidirectional steps along an axis with orientation $\mathbf{e}_{\phi_0}$ are more probable than other directions. This observation for frame-to-frame steps not only persisted when analyzing steps taken within a period of $10\Delta t$, but the modulation was even stronger [Fig. 2(a)]. To verify that the diffusion anisotropy is not caused by contaminations by a few ballistic steps along the cytoskeleton (dispersed in mostly diffusive trajectories), we have also

used a three-state hidden Markov model to complement our trajectory classification [4]. As a result, we observed that the anisotropy also persisted with this separation approach in all cases (Fig. S7 in [4]). Moreover, the anisotropy was seen to persist even when disrupting the cytoskeleton (see below).

As a complement to $p(\phi)$, we also analyzed the angle-resolved average step length $d(\phi) = \langle \Delta r_\phi \rangle_E$. Normalizing $d(\phi)$ by its angle-averaged mean yields the relative step variation, $a(\phi) = d(\phi)/\langle d(\phi) \rangle_\phi$, which showed a very similar modulation as $p(\phi)$, again with equally high peaks at ϕ_0 and $\phi_0 + \pi$ [Fig. 2(b)]. Therefore, peroxisome increments are not only more probable along the axis $\mathbf{e}_{\phi_0}$ but are also longer. In accordance with our observations for $p(\phi)$, the modulation became stronger for steps taken within a period of $10\Delta t$. Thus, (sub)diffusive peroxisome trajectories clearly feature an anisotropic diffusion with larger steps along the axis $\mathbf{e}_{\phi_0}$.

To quantify the anisotropy increase for peroxisomal increments taken in periods $\tau = \Delta t, 2\Delta t, \dots$, we have calculated the ratio $\xi^2(\tau) = \langle x_\parallel^2(\tau) \rangle_{t,E} / \langle x_\perp^2(\tau) \rangle_{t,E}$ of one-dimensional EA-TA-MSDs along and perpendicular to the axis $\mathbf{e}_{\phi_0}$. In line with the data shown in Figs. 2(a) and 2(b), we observed a consistent growth of $\xi^2(\tau)$ for increasing periods τ [Fig. 2(c)]. This result emphasizes the (slight) difference between scaling exponents $\alpha_\parallel$ and $\alpha_\perp$ for the MSDs parallel and perpendicular to $\mathbf{e}_{\phi_0}$ (see the Table in Supplemental Material [4] for all experimental conditions).

Upon normalizing the steps $\Delta x_\parallel$ and $\Delta x_\perp$ by their respective root-mean-squared value within single trajectories (enforcing mean squared step lengths $\langle \chi_\parallel^2 \rangle = \langle \chi_\perp^2 \rangle = 1$ for each trajectory), information on the trajectories' spatiotemporal heterogeneity can be obtained [11]. As a result, we observed that the shape of all PDFs $p(\chi)$ neither depended on the direction ($\parallel$ or $\perp \mathbf{e}_{\phi_0}$) nor on the period τ in which the step was taken [Fig. 2(d)]. However, all PDFs strongly deviated from a normal distribution, indicating that (sub)diffusive peroxisome motion features a considerable spatiotemporal heterogeneity in all directions.

To obtain a meaningful interpretation of our experimental data, we have formulated a simple model [see sketch in Fig. 3(a) and derivations in [4]]. We assume a Gaussian PDF of step increments along and perpendicular to the preferred direction $\mathbf{e}_{\phi_0}$, $\Delta x_\parallel$ and $\Delta x_\perp$, with variances $\sigma_\parallel^2$ and $\sigma_\perp^2$, such that the squared anisotropy $\xi^2 = \sigma_\parallel^2 / \sigma_\perp^2 \geq 1$ is consistent with our above definition of $\xi^2(\tau)$. Transforming Cartesian to polar coordinates yields the PDF $p(\rho, \phi)$ for radial step increments of length ρ along the direction ϕ . From this, the PDF of steps of any length in direction ϕ can be calculated as

$$p(\phi) = \int_0^\infty p(\rho, \phi) \rho d\rho = \frac{\xi}{2\pi} \frac{1 + \tan^2(\phi - \phi_0)}{1 + \xi^2 \tan^2(\phi - \phi_0)}. \quad (1)$$

This expression fits our experimental data very well [Fig. 2(a)], when adjusting the value of ξ to the period τ between successive steps. In addition, one can calculate from Eq. (1) the angular variation of the mean step length [4],

$$d(\phi) = \int_0^\infty p(\rho, \phi) \rho^2 d\rho / p(\phi) = \pi \sigma_\parallel \sqrt{\frac{p(\phi)}{\xi}}. \quad (2)$$

Dividing $d(\phi)$ by its angular average $\langle d(\phi) \rangle_\phi$ yields, as before, $a(\phi)$. The resulting analytical expression fits our experimental data on $a(\phi)$ very well [Fig. 2(b)], without any additional free parameter. To rationalize the experimentally seen growth of $\xi^2 = \sigma_\parallel^2 / \sigma_\perp^2$ [cf. Fig. 2(c)], one may consider an isotropic and antipersistent fractional Brownian motion (FBM) process for $\Delta x_\parallel$ and $\Delta x_\perp$, onto which a one-dimensional Markovian random walk along the direction $\mathbf{e}_{\phi_0}$ is superimposed. While the FBM induces an isotropic subdiffusive motion with $\sigma_\parallel^2 = \sigma_\perp^2 = 2K\tau^\alpha$ and $\alpha < 1$, the additional Markovian steps along $\mathbf{e}_{\phi_0}$ not only elevate the diffusion coefficient K for $\sigma_\parallel^2$ but also perturb the FBM's antipersistent memory. Consequently, a (slightly) higher value of the scaling exponent α along the preferred direction $\mathbf{e}_{\phi_0}$ emerges, leading to a time-dependent increase of ξ^2 .

The observation that each cell features a preferential direction $\mathbf{e}_{\phi_0}$ prompted us to explore the underlying origin. Visual inspection suggested that the long axis of each cell's gyration ellipsoid, $\mathbf{e}_{\theta_0}$ (obtained from the gyration tensor of the convex hull of all peroxisome positions), may correlate with $\mathbf{e}_{\phi_0}$ [cf. sketch in Fig. 3(a)]. Extracting the angle difference $0 \leq |\phi_0 - \theta_0| \leq \pi/2$ between $\mathbf{e}_{\phi_0}$ and $\mathbf{e}_{\theta_0}$, we indeed observed only minor deviations between these two axes, in the range of a few degrees [Fig. 3(b)]. Thus, the global orientation of individual cells appears to determine the preferential direction of (sub)diffusive transport of peroxisomes. This observation is in line with previous findings that endocytosed beads show an elevated mobility along the long cell axis when cells were grown on micro-patterned substrates to increase their aspect ratio [14]. It is, therefore, likely that extended structures like cytoskeletal filaments or the ER network are involved in setting a global director field ϕ_0 for the observed (sub)diffusion anisotropy.

Before testing this hypothesis by disrupting these structures with specific drugs, we have probed the intracellular variation of the (sub)diffusion anisotropy by analyzing its local director field. To this end, we calculated for all (sub)diffusive trajectories within a cell all frame-to-frame increment vectors in polar coordinates, $\mathbf{r}(t + \tau) - \mathbf{r}(t) \mapsto (\Delta r, \varphi)$ with $\tau = \Delta t$. The resulting angles φ were assigned to the respective average positions $\mathbf{r}^*(t; \tau) = [\mathbf{r}(t + \tau) + \mathbf{r}(t)]/2$. Partitioning the plane into square bins of edge length $1 \mu\text{m}$, the positions $\mathbf{r}^*(t; \tau)$ were discretized, and data for φ were assigned to the respective bins, yielding N_b angles in bin b . To exclude statistically

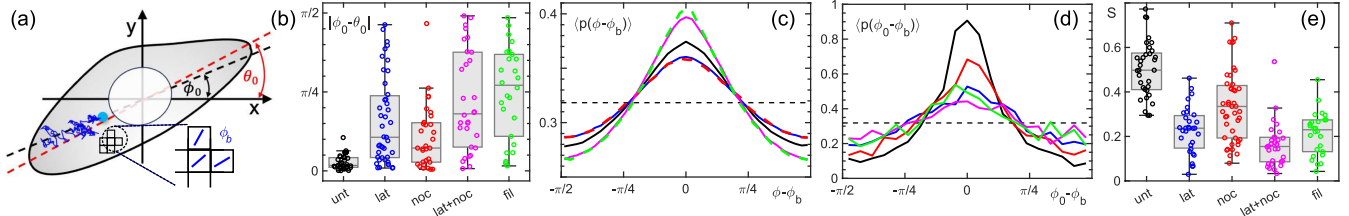


FIG. 3. (a) Sketch of the orientations of trajectories and the cell’s long axis. Squares indicate the bins for defining the local directors ϕ_b [Eq. (3)]. (b) The angle difference $|\phi_0 - \theta_0|$ between the preferential direction of peroxisome motion, $\mathbf{e}_{\phi_0}$, and the long cell axis, $\mathbf{e}_{\theta_0}$, is on average only 5° in untreated cells (“unt,” black symbols). For latrunculin- and nocodazole-treated cells (“lat” and “noc,” blue and red symbols), the deviations are considerably larger, but are on average only about 30° . Upon treatment with latrunculin and nocodazole (“lat + noc,” magenta) or with filipin (“fil,” green), both of which disrupt the ER, the angle difference grew to a mean of 45° , indicating a strong randomization of the axes’ relative orientation. (c) Cell-ensemble averages of $p(\phi - \phi_b)$ feature a significant modulation and, hence, a local anisotropic diffusion for all conditions (same color code as before). Black dashed line: expectation for isotropic motion. (d) The ensemble-averaged PDF $\langle p(\phi_0 - \phi_b) \rangle$ indicates a strong alignment of local directors ϕ_b to a global director ϕ_0 for untreated cells. Although softened when disrupting the cytoskeleton or the ER, a marked modulation remains. (e) The nematic order parameter S quantifies the latter, indicating a substantial alignment of ϕ_b and ϕ_0 in untreated cells, whereas the disruption of the ER or cytoskeletal elements reduces the alignment (cf. also main text).

poor bins from subsequent analyses, we only considered bins with $N_b \geq 5$. With this, we defined a local director field ϕ_b that determines the locally preferred direction in bin b [cf. Fig. 3(a)]:

$$Q_b = \frac{1}{N_b} \sum_{k=1}^{N_b} e^{2i\varphi_k} =: q_b e^{2i\phi_b}. \quad (3)$$

Given that we are dealing with a nematic symmetry, angle shifts $\varphi \rightarrow \varphi + \pi$ must not change the order parameter, hence the factor 2 in the exponent. An example for the local director field ϕ_b and for the associated scalar order parameter q_b (which measures how much peroxisomal steps align with the local director ϕ_b) is shown in Fig. S1 in Supplemental Material [4]. Despite being low, the scalar order parameter values $q_b \approx 0.1$ were significantly different from the null result obtained for random phases φ_k , and they are in line with previous reports on lyotropic nematic phases [15,16]. Moreover, despite the low values of q_b , the field of local directors showed a strong alignment, as quantified by the associated nematic order parameter (see next paragraph). To account for both facets, we will use the term “weakly nematic” in the remainder.

To quantify the robustness of our findings, beyond the single cell example, we analyzed an ensemble of $n = 33$ untreated cells with $m = 52\,818$ trajectories. As a result, we observed that (sub)diffusive step increments in all cells showed a clear anisotropy, highly similar to Fig. 2. In particular, the (sub)diffusive steps in all cells nicely followed a local director ϕ_b , indicated by a marked modulation in the PDF $p(\phi - \phi_b)$ [Fig. 3(c)]. In addition, the local directors ϕ_b closely followed the cell-specific global director, ϕ_0 , as evidenced by the ensemble-average PDF $\langle p(\phi_0 - \phi_b) \rangle$ [Fig. 3(d)] and by the corresponding nematic order parameter $S = \langle \cos(2[\phi_b - \phi_0]) \rangle$ that assumed values around $S = 0.5$ [Fig. 3(e)]. Together with

the correlation $\phi_0 \approx \theta_0$ [Fig. 3(b)], this confirms an anisotropic (sub)diffusion of peroxisomes also for an ensemble of cells. As in individual cells, the anisotropy has the same local and global preference axis that correlates with the long axis of the cell. Separating (sub)diffusive step increments with a three-state hidden Markov model, as an alternative to the selection via α and C , yielded highly similar results [4]. We, therefore, conclude that the observed anisotropy is very similar at all cell loci, suggesting that the diffusion anisotropy emerges from local structures that adopt a cell-wide directional preference.

Next, we inspected ensembles of cells in which cytoskeletal filaments or the ER were disrupted by specific drugs [4]. Analyses of single example cells (corresponding to Figs. 1 and 2) are summarized in Figs. S2–S5 in Supplemental Material [4]. Given that actin filaments are known to be key for cell adhesion and shape, even aligning with the major cell axis (see, e.g., Ref. [17]), we anticipated changes in the (sub)diffusion anisotropy when disrupting actin filaments by the established drug latrunculin A [18] ($n = 50$ cells, $m = 33\,082$ trajectories). Somewhat unexpectedly, however, the modulation in $p(\phi - \phi_b)$ was only softened slightly [Fig. 3(c)], indicating again a strong anisotropic (sub)diffusion with local directors ϕ_b . As compared to untreated cells, however, the alignment of the local directors to a global direction was less strong, as evidenced by the broadened PDF $\langle p(\phi_0 - \phi_b) \rangle$ and a reduced nematic order parameter S [Figs. 3(d) and 3(e)]. In addition, the correlation of $\mathbf{e}_{\phi_0}$ and $\mathbf{e}_{\theta_0}$ was less strong [Fig. 3(b)]. Treatment with latrunculin A was also seen to slightly reduce the fraction of trajectories with a superdiffusive signature and to alter the PDF of step lengths between successive frames, $p(\Delta r)$, for (sub)diffusive trajectories, featuring markedly larger values and an increased mean as compared to untreated cells (Fig. S6 in Supplemental Material [4]). Thus, our data provide

evidence that actin filaments are not the dominant cause of the observed anisotropic (sub)diffusion of peroxisomes. Rather, intact actin filaments appear to hamper the motion of peroxisomes.

We then applied the drug nocodazole via an established protocol [19,20] to disrupt all microtubules ($n = 31$ cells, $m = 67\,471$ trajectories). Microtubules are best known as filaments along which organelles are transported. In line with this notion, we observed that almost no trajectories with a superdiffusive signature remained (with values for α and C near the selection threshold). Still, we observed a similar modulation in $p(\phi - \phi_b)$ as for latrunculin-treated cells [Fig. 3(c)] and an even better alignment of the local directors to a global direction [Figs. 3(d) and 3(e)]. Therefore, even in the absence of microtubules, the modulation remained significant, and also the correlation between $\mathbf{e}_{\phi_0}$ and $\mathbf{e}_{\theta_0}$ remained similar to latrunculin-treated cells [Fig. 3(b)]. The PDF of (sub)diffusive steps, $p(\Delta r)$, hardly deviated from that of untreated cells (Fig. S6 in Supplemental Material [4]). From these data, we conclude that microtubules might contribute to the observed anisotropy, but they are not the sole or even dominant cause for its emergence.

Combined treatment with nocodazole and latrunculin A ($n = 30$ cells, $m = 25\,996$ trajectories), which cells tolerate only for a very limited time before dying off, resulted in a further dealignment of local and global directors and a further randomization between ϕ_0 and θ_0 (Fig. 3). On local scales, however, the (sub)diffusion anisotropy persisted, and the modulation in $p(\phi - \phi_b)$ was even slightly enhanced (Fig. 3). Therefore, the cytoskeleton cannot be held responsible for the locally occurring anisotropy, but the data suggest, at first glance, that the combined cytoskeleton is the major organizer for aligning local directors to each other. Yet, we also observed that the ER was severely compromised in these cells. Hypothesizing that the ER is the important structure, rather than the filaments, we selectively disrupted the ER network by applying filipin [21]. The result ($n = 26$ cells, $m = 7405$ trajectories) was very similar to the combined treatment with nocodazole and latrunculin A (Fig. 3), providing strong evidence that the ER, rather than the cytoskeleton, is the major organizer for aligning the local directors ϕ_b to a global director ϕ_0 . Again, the modulation in $p(\phi - \phi_b)$ was higher than in untreated cells, indicating a substantial (sub)diffusion anisotropy.

We speculate that this enhancement is due to the fragmentation of the ER into small membrane structures that are scattered across the cytoplasm, complementing already existing membrane structures, such as mitochondria or endosomes. All of these interfaces may locally block and guide movements of neighboring particles, hence enforcing a local anisotropy for diffusional motion of neighboring particles, akin to the properties of a weakly nematic fluid. Without a cell-spanning structure, these

constraints remain local and do not get aligned, whereas an intact ER may organize local director fields to a global preference direction. The situation is hence reminiscent of the initial magnetization of a ferromagnetic material: spontaneously formed ferromagnetic domains (here: weakly nematic regions with director ϕ_b) need to be aligned by an external field (here: the ER) to form a preferred direction on macroscopic scales (here: ϕ_0). In any case, cytoskeletal filaments are dispensable for local anisotropy and also appear unessential for the emergence of a global direction for anisotropic (sub)diffusion. Our findings are, hence, very different from earlier reports on anisotropic diffusion during mitosis [22] and in elongated cells on micropatterned surfaces [14]. Based on these considerations, we expect that not only peroxisomes but virtually all particlelike structures in the cytoplasm will exhibit a similar anisotropy signature, organized by the ER network.

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Data availability—The data that support the findings of this article are not publicly available. The data are available from the authors upon reasonable request.

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