Spatial cytoskeleton organization is optimized for targeted intracellular cargo transport

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The efficiency of intracellular transport of cargo from specific source to target locations is strongly dependent upon molecular motor assisted motion along cytoskeleton filaments, microtubules and actin filaments. Radial transport along microtubules and lateral transport along the filaments of the actin cortex underneath the cell membrane are characteristic for cells with a centrosome. Here we show that this specific filament organization for ballistic transport in conjunction with intermittent diffusion realizes a spatially inhomogeneous intermittent search strategy that is in general optimal for small thicknesses of the actin cortex. We prove optimality in terms of mean first passage times for three different, frequently encountered intracellular transport tasks: i) the narrow escape problem (e.g. transport of cargo to a synapse or other specific region of the cell membrane, ii) reaction kinetics enhancement (e.g. binding of a mobile particle with a immobile or mobile target within the cell, iii) the reaction-escape problem (e.g. release of cargo at a synapse after intracellular vesicle pairing. The results indicate that living cells realize optimal search strategies for various intracellular transport problems economically through a spatial cytoskeleton organization that involves only a narrow actin cortex rather than a cell body filled with randomly oriented actin filaments.

The cytoskeleton of living cells is a self-organizing filamentous network that shapes the mechanical and rheological characteristics of the cell and co-ordinates cargo transport between different cellular regions. Intracellular transport of particles equipped with one or several motors switches between two modes of motion: free diffusion within the cytosol and ballistic motion during intermittent bindings of the motor(s) to a cytoskeleton filament. Typically cargo, like proteins, vesicles, and other organelles, is produced or emerges in one region of the cell and is needed in some other region or has to fuse with a reaction partner being produced somewhere else. In the absence of a direct connection between origin and destination the transport is a stochastic process with random alternations between ballistic and diffusive motion, which is denoted as intermittent search. A particular set of parameters defining the stochastic process, like the switching rate between ballistic and diffusive transport, represents an intermittent search strategy and it has been shown that an optimal choice of this parameter enhances the search efficiency in a homogeneous and isotropic environment, i.e. under the assumption of a constant density of filaments with no preferred direction. Real cell cytoskeletons display a complex spatial organization, which is neither homogeneous nor isotropic. For instance in cells with a centrosome the microtubules emanate radially from a microtubule organizing center (MTOC) and actin filaments form a thin cortex underneath the plasma membrane with broad distribution of directions again centered around the radial direction, see Fig.1 for a sketch. Since the MTOC is frequently located close to the nucleus, transport of cargo between plasma membrane and nucleus, as for instance necessary for the establishment of synaptic junctions, appears to be facilitated by this cytoskeleton organization. In essence the specific spatial organization of the cytoskeleton filaments represents, in connection with intracellular transport, an intermittent search strategy, probably optimized for specific frequently occurring tasks, but less well suited for others. It is unknown how much the efficiency gain or loss of such a spatially inhomogeneous intermittent search strategy for a particular task actually is, in particular in comparison with homogeneous search strategies. In this letter we analyze this question with the help of a model for intermittent dynamics in spatially inhomogeneous environments.

A search strategy that idealizes the cytoskeleton structure in a spherical cell of radius as sketched in Fig.1 consists of microtubule filaments emanating radially from the MTOC in the cell center and randomly oriented actin filaments.
filament in a cortex of width $\Delta$ underneath the plasma membrane. Mathematically this filament density is defined by the probability density $\rho_0(\mathbf{r})$ to switch at position $\mathbf{r}$ from the diffusive mode to a ballistic motion in the direction $\Omega = (\phi, \theta)$:

$$
\rho_0(\mathbf{r}) = \begin{cases} 
p \delta(\Omega - \Omega(\mathbf{r})) + (1-p) \delta(\Omega - \Omega(-\mathbf{r})), & 0 < |\mathbf{r}| < R - \Delta,  
1/(4\pi), & R - \Delta < |\mathbf{r}| < R 
\end{cases}
$$

(1)

The MTOC is assumed to be located in the origin ($\mathbf{r} = 0$), $\Omega(\mathbf{r})$ is the solid angle of the position vector $\mathbf{r}$, and $p$ is the probability to move radially outwards (microtubule plus direction) and $1 - p$ to move radially inward (microtubule minus direction). A radial dependence of the microtubule density is neglected here. Note that for $\Delta = R$ one obtains the filament density considered in [10].

We model the intracellular motion of cargo by an intermittent search process [9, 10, 12, 13], in which a particle performs random motion in two alternating modes: Brownian motion with diffusion constant $D$, and ballistic motion with velocity $\mathbf{v}$. Transitions between the two modes occur stochastically with rate $k$ and $k'$, respectively. Mathematically the underlying stochastic process is described by a Fokker-Planck equation for the time evolution of the probability distribution $P_0(\mathbf{r}, t)$ for the freely diffusing particle and $P_{\Omega}(\mathbf{r}, t)$ for the particle that moves ballistically with velocity $|\mathbf{v}|$ in the direction $\Omega$:

$$
\frac{\partial}{\partial t} P_0(\mathbf{r}, t) = D(\mathbf{v} \cdot \mathbf{r}) - k P_0(\mathbf{r}, t) + k' \int d\Omega P_{\Omega}(\mathbf{r}, t) 
$$

(2)

$$
\frac{\partial}{\partial t} P_{\Omega}(\mathbf{r}, t) = -\nabla (\mathbf{v} P_{\Omega}(\mathbf{r}, t)) + k \rho_0(\mathbf{r}) P_0(\mathbf{r}, t) - k' P_{\Omega}(\mathbf{r}, t) .
$$

$k$ and $k'$ are attachment and detachment rates and we assume the modulus of the velocities $v = |\mathbf{v}|$ to be constant throughout the following. At time $t = 0$ we assume the particle to be at position $\mathbf{r}_0$ and in the diffusive mode: $P_0(\mathbf{r}, t = 0) = \delta(\mathbf{r} - \mathbf{r}_0)$, $P_{\Omega}(\mathbf{r}, t = 0) = 0$. Apart from the stochastic detachment with rate $k'$ a ballistically moving particle switches automatically to the diffusive mode at the MTOC ($|\mathbf{r}| = 0$) since microtubules end here, at the inner border of the actin cortex ($|\mathbf{r}| = R - \Delta$) since actin filaments end here, and at the cell membrane ($|\mathbf{r}| = R$) since all filaments end here. Diffusing particles are reflected at the cell membrane ($|\mathbf{r}| = R$), apart from small regions in the cell membrane in exit problems, where absorbing boundary conditions are applied. In the following we use rescaled dimensionless spatial and temporal coordinates $\mathbf{r} = \mathbf{r}/R$ and $t = vt/R$, and the rescaled parameters $\bar{D} = D/vR$, $\bar{k} = Rk/v$ and $\bar{k}' = Rk'/v$.

The efficiency of a search strategy, or a specific filament density $\rho_0(\mathbf{r})$ is measured in terms of a mean first passage time (MFPT) with respect to the events defined by the different search problems that we consider now. We use an efficient event-driven kinetic Monte Carlo algorithm [14] to generate the stochastic process underlying [2] and calculate the mean MFPT $\bar{T}$ by sampling the generated process. We use of the order of $10^6$ realizations of the process for each parameter value such that the relative statistical error for the MFPT is significantly lower than 0.5%.

**Narrow escape problem:** First we consider intracellular transport of cargo from an arbitrary position within the interior of the cell, typically from a location close to the nucleus, to a specific area on the cell boundary, the plasma membrane. A concrete example involves the directed secretion by immune cells require the formation of an immunological synapse [15, 16], and transport of secretion material towards the synapse involves the cytoskeleton [17, 18]. The stochastic search for a specific small area on the boundary of a search domain is reminiscent of the so-called narrow escape problem [19, 20]. Here we ask, whether the specific organization of the cytoskeleton as sketched in Fig.1 has the potential to solve the narrow escape problem faster, and if yes, how much faster.

To answer this question we compute the MFPT for various parameters $k$, $k'$, and $\Delta$ in [2] and [1] for small escape regions of azimuth angle $\vartheta_{\text{abs}} = \arcsin(1/7) \approx 0.1433$ (corresponding to an absorbing area of only 0.51%
of the total spherical surface) as sketched in Fig. 2a. In order to demonstrate the efficiency of a spatially inhomogeneous filament density (corresponding to $0 < \Delta < R$ in eq. (1)) we first determine the optimal attachment and detachment rates, $k_{\text{opt}}(D)$ and $k'_{\text{opt}}(D)$, respectively, for a homogeneous filament density ($\Delta = 1$). Fig. 2b shows the MFPT as a function of the rates $k$ and $k'$ for $D = 0.05$. The optimal detachment rate $k'_{\text{opt}}$ is zero, which holds for all diffusion constants $D$. $k_{\text{opt}}(D) = 0$ means that uninterrupted ballistic transport to the cell membrane is optimal for the narrow escape problem, which is plausible, since the target area is on the membrane. The optimal attachment rate $k_{\text{opt}}$ decreases with increasing diffusion constant $D$ (Fig.S1a in [21]). For small diffusion constants $D < 0.1$ the homogeneous intermittent search is always more efficient than the pure diffusive search. The MFPT of the latter diverges for vanishing diffusion constant as $T_{\text{diff}} \approx 7.62/D$, for $\theta_{\text{abs}} = \arcsin(1/7)$ [22], whereas the MFPT for homogeneous intermittent search $T_{\text{opt}}$ stays finite (Fig.S1a in [21]).

Next we take the optimal values $k_{\text{opt}}(D)$ and $k'_{\text{opt}}(D)$ for $\Delta = 1$, and vary the width of the actin cortex $\Delta$, but fix the value of $p$ in (1) to one (only outward radial transport). The result is shown in Fig. 2b: For small values of $D < 0.02$ it is possible to enhance the efficiency compared to the case of a spatially homogeneous filament direction density ($\Delta = 1$). For larger values ($0.02 < D < 0.06$) the optimal strategy for the rates $k_{\text{opt}}(D)$ is a homogeneous one. However, allowing for rates different from $k_{\text{opt}}(D)$ superior inhomogeneous search strategies exist: Minimizing the MFPT as a function of $k$ and $\Delta$ simultaneously the optimal strategy has a small, non-vanishing value for $\Delta$ (Fig.S1b in [21]) for $D = 0.04$. For $0.06 < D < 0.1$ a search strategy with a fully polarized network ($\Delta = 0$) is optimal, and even more efficient general distributions $\rho(r)$ for this case exist. Finally, even for fixed attachment and detachment rates, $k$ and $k'$, the MFPTs are minimized for small, non-vanishing values of $\Delta$ (Fig.S1c in [21]).

Enhanced reaction kinetic: Next we consider the enhancement of the reaction kinetics between two reaction partners by motor assisted ballistic transport. It has already been demonstrated that spatially homogeneous and isotropic intermittent search strategies can decrease mean first passage times substantially [9, 10]. Such intermittent search strategies are only realized in those parts of a biological cell, where cytoskeleton filaments are homogeneously and isotropically distributed, which is certainly not true for the whole cell body. Therefore we ask, how efficient a realistic spatial organization of the cytoskeleton can actually be in particular for a search for an immobile intracellular reaction partner that is preferentially located in a specific sub-volume of the cell (if both reaction partners are mobile an obvious suitable strategy would be to transport both of them towards the MTOC to bring the into contact). Fig. 3a shows a sketch of the search process for the case of an immobile target at position $r_{\text{tar}}$. When the searcher is in the diffusive mode and its position $r$ comes closer to the target than $|r - r_{\text{tar}}| \leq d$ it reacts with the target and the search is finished. The target position $r_{\text{tar}}$ with $0 \leq r_{\text{tar}} \leq 1 - d$ will be either homogeneously distributed or it is predominantly located close to the center $r_{\text{tar}} \leq 1/2$ with probability $w$. Fig. 3b shows the result for the MFPT for the spatially homogeneous filament direction density ($\Delta = 1$) and the parameters $D = 1/300$ and $d = 0.025$ as a function of the attachment and detachment rates $k$ and $k'$. In contrast to the narrow escape problem the optimum is now not at $k' = 0$ any more, which is reasonable, since the target is not located on the boundary. The concentration of the target towards the center ($w = 0.9$) does not change the MFPT significantly even for a rather large value of $d$ (see Fig.S2a in [21]).

Again we take the optimal values $k_{\text{opt}}(D)$, $k'_{\text{opt}}(D)$ from $\Delta = 1$ and calculate with these rates the MFPT.
for the inhomogeneous filament density for fixed values of outward transport probability \( p \) as a function of the cortex width \( \Delta \). The result, shown in Fig.3, demonstrates that again a thin cortex \( \Delta \ll 1 \) minimizes the search times for \( p = 1/2 \). Moreover, although the target is located with a high probability close to the center \( (w = 0.75 \text{ and } 0.9) \) the MFPT is not minimized for small values of \( p \), corresponding to preferential radial transport towards the center, but for \( p \) close to 1/2. A similar observation can be made if we fix the cortex width to a small value, \( \Delta = 0.1 \), and vary \( p \), instead, as shown in Fig.3b: the minimum MFPT is attained for values of \( p \) around 1/2, even for large \( w \) and also for fixed non-optimal rates \( k, k' \). These results are confirmed for larger values of the parameters: \( D = 0.01 \) and \( d = 0.1 \) (see Fig.S2b and Fig.S2c in [21]).

**Reaction-escape problem:** Finally we consider the combination of the reaction and escape problem, where cargo has first to bind to a reaction partner before it can be delivered or dock at a specific area in the cell boundary as, for instance, a synapse. A prominent example is the docking of lytic granules at the immunological synapse of cytotoxic T-lymphocytes that requires the pairing with CD3 endosome beforehand [23]. Vesicles containing lytic granules have a low docking probability at the IS, whereas vesicles loaded with CD3 receptors have a high docking probability. Apparently it represents an advantageous strategy to guarantee the delivery of cytotoxic cargo exclusively to the IS to bind to the T-cell receptor CD3 first and we ask how the spatial organization of the cytoskeleton supports the efficiency of this strategy.

Fig.4a shows a sketch of the stochastic process, now involving two particles, a searcher and a target, both moving as the single particle in the preceding two models with additional boundary conditions: The searcher and the target react and build a pair once they get closer than a distance \( d \) and both being in the diffusive mode, the MFPT for the reaction event is denoted \( T_{\text{reac}} \). The escape area is only absorbing for the pair, the MFPT for the pair escaping the cell is denoted \( T_{\text{esc}} \). The total MFPT for the initially two particles starting at random positions until the escape of the paired particle is \( T = T_{\text{reac}} + T_{\text{esc}} \).

Fig.4b shows \( T \) for the homogeneous filament density \( (\Delta = 1) \) as a function of the rates \( k, k' \) for \( D = 0.01, d = 0.1, \vartheta_{\text{asso}} = \arcsin(1/7) \). \( T_{\text{reac}} \) and \( T_{\text{esc}} \) are shown separately in Fig.S3a in [21]. For the reaction part the MFPT \( T_{\text{reac}} \) is minimized by a non-vanishing detachment rate \( k' \), whereas the escape MFPT \( T_{\text{esc}} \) is again minimized for \( k' = 0 \). The sum of both, \( T \), is also minimized by \( k' = 0 \). It should be noted that for a purely diffusive target the total MFPT is minimized by a non-vanishing rate \( k' = 0 \). In Fig.4c we show the MFPTs for the inhomogeneous filament density eq. (1) as a function of the cortex width \( \Delta \), with the optimal rates for the homogeneous case for \( D = 1/300 \) for \( p = 1/2 \) (outward and inward radial transport equally probable) and for \( p = 1 \) (only outward radial transport). Data for \( D = 0.01 \) are shown in Fig.S3b in [21]. One observes that for the smaller diffusion constant the symmetric radial transport \( (p = 1/2) \) reduces the total MFPT by 70% for small cortex width. Also for the larger diffusion constant one can reduce \( \Delta \) down to zero without changing the total MFPT substantially. Even if one fixes the attachment and detachment rates to non-optimal values the total MFPT is again reduced by at least 50% for small cortex widths (see Fig.S3c in [21]).

FIG. 4. a) Sketch of the reaction-escape process, involving two particles, starting diffusively at random positions \( F_0 \) (grey trajectory) and \( F_1 \) (green trajectory). Before being allowed to leave at the escape area, they have to react first. They react when coming closer than a distance \( d \) and form a pair (brown trajectory), which will be absorbed at the escape region represented by the dotted segment on the cell boundary. b) Homogeneous filament density \( (\Delta = 1) \): \( T \) as a function of \( k \) and \( k' \) for \( D = 0.01, d = 0.1, \vartheta_{\text{asso}} = \arcsin(1/7) \). The red dot represents the minimum. c) Inhomogeneous filament density: The MFPTs \( T_{\text{reac}}, T_{\text{esc}}, T \) for the optimal attachment / detachment rates \( (k, k') \) as a function of the actin cortex width \( (\Delta) \) for symmetric radial microtubule transport \( (p = 1/2) \) in the left panel, and purely outward transport microtubule transport \( (p = 1) \) in the right panels for \( (D = 1/300, d = 0.025) \).

The spatial organization of the cytoskeleton of cells with a centrosome does not only minimize the characteristic time necessary for various transport tasks but does so in an economic way: instead of supporting a resource demanding isotropic homogeneous filament network it is sufficient, and almost always even more efficient, to establish just a thin actin cortex underneath the cell membrane. Our analysis supports this conclusion rigorously and quantitatively for spherical cells with a centrosome but it is plausible that the spatial organization of the cytoskeleton is universally optimized for transport and
search problems in other cell types.
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FIG. S1. **Narrow escape problem.** a) MFPT for the purely diffusive, $T_{\text{diff}}$ (red line), and intermittent search, $T_{\text{opt}}$ (green line), with a homogeneous filament density ($\Delta = 1$) with optimal transition rates, as functions of the diffusion constant $\tilde{D}$. The optimal attachment rate $\tilde{k}_{\text{opt}}(\tilde{D})$ is represented by the blue line (and right y-axis), the optimal detachment rate is $\tilde{k}'_{\text{opt}} = 0$. b) MFPT as a function of $\tilde{k}$ and $\Delta$ for $\tilde{D} = 0.04$, $\tilde{k}' = 0$ and $p = 1$. The red dot indicates the global minimum, and the green dot (top left) indicates the minimum for the homogeneous case ($\Delta = 1$). c) $\tilde{T}$ as a function of $\Delta$ for fixed rates $\tilde{k} = \tilde{k}' = 10$ for different $\tilde{D}$ and $p = 1$. 
FIG. S2. **Enhanced reaction kinetics.**

- **b)** Homogeneous filament density: MFPTs as a function of \( \tilde{k} \) and \( \tilde{k}' \) for a spatially homogeneous filament density (\( \tilde{\Delta} = 1 \)), for (left) \( \tilde{D} = 0.01, \tilde{d} = 0.1 \), homogeneously distributed target position \( \tilde{r}_{\text{tar}} \); (right) \( \tilde{D} = 0.01, \tilde{d} = 0.1 \), target position \( \tilde{r}_{\text{tar}} \leq 0.5 \) with probability \( w = 0.9 \)

- **c)** MFPT for the inhomogeneous filament density with the optimal rates \( \tilde{k}_{\text{opt}}(\tilde{D}, \tilde{d}) \) from the homogeneous case \( \tilde{\Delta} = 1 \) as function of \( \tilde{\Delta} \) for different values of the forward radial transport \( p \) and different target positions close to the center \( w \).

- **d)** MFPT as in c) but now with fixed width \( \tilde{\Delta} = 0.1 \) as function of the forward probability \( p \) for different fixed rates \( k \) and \( k' \) different values of \( w \).
FIG. S3.  Reaction-escape problem. a) Homogeneous filament density ($\Delta = 1$): MFPTs for reaction ($\tilde{T}_{\text{reac}}$, left), pair escape ($\tilde{T}_{\text{esc}}$, right) as a function of $\tilde{k}$ and $\tilde{k}'$ for $\tilde{D} = 0.01, \tilde{d} = 0.1, \vartheta_{\text{abs}} = \arcsin(1/7)$. The red dots represents the minimum. b) Inhomogeneous filament density: The MFPTs $\tilde{T}_{\text{reac}}, \tilde{T}_{\text{esc}}, \tilde{T}$ for the optimal attachment / detachment rates ($\tilde{k}, \tilde{k}'$) from figure Fig.2b) as a function of the actin cortex width ($\Delta$) for symmetric radial microtubule transport ($p = 1/2$) in the left panel, and purely outward transport microtubule transport ($p = 1$) in the right panel. c) The MFPTs $\tilde{T}_{\text{reac}}, \tilde{T}_{\text{esc}}, \tilde{T}$ for fixed, non-optimal attachment / detachment rates $\tilde{k} = 5, \tilde{k}' = 10$ for ($\tilde{D} = 1/300, \tilde{d} = 0.025$).