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Intracellular fluid flow in rapidly moving cells

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Abstract

Cytosolic fluid dynamics have been implicated in cell motility^{1–5} because of the hydrodynamic forces they induce and because of their influence on transport of components of the actin machinery to the leading edge. To investigate the existence and the direction of fluid flow in rapidly moving cells, we introduced inert quantum dots into the lamellipodia of fish epithelial keratocytes and analysed their distribution and motion. Our results indicate that fluid flow is directed from the cell body towards the leading edge in the cell frame of reference, at about 40% of cell speed. We propose that this forward-directed flow is driven by increased hydrostatic pressure generated at the rear of the cell by myosin contraction, and show that inhibition of myosin II activity by blebbistatin reverses the direction of fluid flow and leads to a decrease in keratocyte speed. We present a physical model for fluid pressure and flow in moving cells that quantitatively accounts for our experimental data.

Various indirect results^{4,6} suggest that fluid influx at the leading edge could play an active part in actin-based cell motility by generating hydrodynamic forces that oppose the membrane load and thus increase the rate of actin polymerization at a protruding edge. Notably, the expression of aquaporins, which are highly enriched at the leading edge and increase water permeability of the membrane, accelerates motility and increases the metastatic potential of melanoma cells^{4,7}. Alternatively, it has been suggested that intracellular fluid flow towards the leading edge might contribute to motility by expediting transport of actin and other soluble proteins to the leading edge⁸. Direct measurements of fluid flow in the lamellipodia of moving cells are

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AUTHOR CONTRIBUTIONS

K.K. and J.A.T. conceived and designed the experiments; K.K. performed the experiments and analysed the data; A.M. and K.K. developed the model; P.T.Y. made the initial observation of enhancement of large probes at the leading edge; A.K. contributed to the single-particle tracking experiments; K.K., A.M., P.T.Y. and J.A.T. discussed the results and wrote the paper.

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COMPETING INTERESTS

The authors declare that they have no competing financial interest.

lacking, as methods for measuring intracellular flow⁹ typically use large organelles or particles (hundreds of nm to a few μm in diameter), which cannot penetrate the dense lamellipodial actin meshwork characterized by a pore size of about 50 nm^{10,11}. As shown below, smaller particles (<50 nm) that can penetrate the dense actin meshwork diffuse rapidly, severely limiting the sensitivity of fluid flow measurements based on single-particle tracking in live cells. To overcome this difficulty, we introduced a new approach in which the direction and magnitude of fluid flow are determined from measurements of the steady-state size-dependent spatial distribution of inert probes.

Fish keratocytes are a simple and widely used model system for studying the dynamics of the motility process. They are among the fastest moving animal cells, with average speeds of about $0.3 \mu\text{m s}^{-1}$, yet their motion is extremely persistent, with hardly any change in cell shape, speed or direction over many minutes^{12,13}. In these cells, any intracellular fluid flow associated with motility should be persistent because of the steady-state nature of their motion. Furthermore, the broad, flat and extremely thin ($\sim 100\text{--}200 \text{ nm}$)¹⁰ lamellipodia of these cells alone are sufficient for persistent motility¹². We set out, therefore, to measure the fluid dynamics within the lamellipodia of moving keratocytes. We introduced fluorescent tracers into the cytosol as probes for the fluid dynamics. For these tracers to faithfully report the motion of the fluid phase of the cytoplasm, they must have minimal nonspecific interactions with the surrounding cellular milieu, in particular the actin meshwork in the lamellipodium. Polyethyleneglycol coating has been shown to be optimal in reducing nonspecific interactions in both *in vivo*¹⁴ and *in vitro* actin networks¹⁵, so we chose methoxypolyethyleneglycol-coated quantum dots (QDs) as probes.

We followed the motion of individual 655QDs in live keratocytes (Fig. 1) and observed rapid movement in the lamellipodia, characterized by an average apparent diffusion coefficient of $D = 1.15 \pm 0.5 \mu\text{m}^2 \text{ s}^{-1}$ (mean $\pm$ s.d., $D = \text{MSD}(t)/4t$ with $\text{MSD}(t) = \langle \delta r(t)^2 \rangle$ where $\delta r(t)$ is the QD displacement during the time interval $t = 0.3 \text{ s}$; $n = 37$ cells). We found considerable cell-to-cell variability in the rate of apparent QD diffusion (Fig. 1c–e), probably reflecting differences in the density and organization of the lamellipodial actin meshwork among cells^{16,17}. Fluid flow should induce a bias in the motion of tracer particles as measured by single-particle tracking. In principle, even a small persistent flow could be detected by averaging over a large number of particles and/or over extended periods of time. However, in practice, several limitations set a lower limit on the magnitude of fluid flow that we can detect in a moving keratocyte by single-particle tracking¹⁸ (Supplementary Information, Supplementary Text B2). Within this detection limit ($\sim 0.2 \mu\text{m s}^{-1}$) we did not observe a persistent bias in QD motion in the cell frame of reference in keratocytes ($n = 10$ cells; Supplementary Information, Fig. S1).

To increase the sensitivity of our fluid flow measurements in the lamellipodium, we turned to measuring flow-induced effects on the size-dependent distribution of inert probes. Consider a simplified case in which the lamellipodium is approximated as a thin rectangular box, and persistent fluid flow occurs along its posterior–anterior axis. We assume that the membrane water permeability is concentrated at the leading edge, as has been shown in other cell types^{4,5}. In this case, the fluid flow V_f will be uniform, and the probe distribution $n(x)$ must

satisfy the steady-state 1D diffusion-convection equation, $\frac{d^2 n}{dx^2} = -\frac{V_f}{D} \frac{dn}{dx}$, giving an exponential probe distribution, $n(x) \propto \exp(-x/L)$, where x is the distance from the leading edge, D is the diffusion coefficient of the probe and $L = D/V_f$. Thus, forward-directed fluid flow in the cell frame of reference will generate an accumulation of probe near the leading edge, whereas rearward flow will cause a depletion of probe at the leading edge. The extent of probe accumulation depends on the relative magnitude of flow and diffusion (Fig. 2a, b), so it should be evident for large probes ($D \sim L_0 V_f$; $L_0 \sim 10 \mu\text{m}$ is the lamellipodial length), and negligible

for rapidly diffusing small probes ($D \gg L_0 V_f$). These results are qualitatively unchanged if the movements of the particles are not purely diffusive, but have slightly subdiffusive characteristics (Supplementary Information, Supplementary Text C). Our fluid flow measurements are based on the steady-state nature of the flow. The typical timescale required for equilibration of the probe distribution is $L_0/D \sim 1$ min, so our technique is applicable to cells, such as keratocytes, that show persistent behaviour over such timescales.

To measure fluid flow in moving keratocytes, we introduced large inert probes into the cytoplasm of live cells and determined their distribution in the lamellipodium, using ratio imaging with a small probe that acted as a volume marker to correct for thickness variations. In addition to the QDs, we also used large fluorescent dextrans, which have been previously used to characterize size-dependent intracellular motion in live cells¹⁹, and have little nonspecific interaction. We tested several large probes (diameter >20 nm; 655QDs (30.5 ± 1 nm), 565QDs (23 ± 2 nm), 500 kD dextran) in various combinations with several small probes (diameter <1 nm; AlexaFluor488 free dye, AlexaFluor594 free dye, 3K dextran). Irrespective of the combination of probes used, we found enhancement of large probes relative to small probes towards the leading edge (Fig. 2c, d; Supplementary Information, Figs S2, S3), consistent with a forward-directed flow in the cell frame of reference, from the cell body towards the leading edge.

To estimate the magnitude of fluid flow, we measured the distribution of the 655QDs density, for which we had already measured the apparent diffusion coefficient (Fig. 1). The ratio density along a cross-section perpendicular to the leading edge (Fig. 2c, bottom panel) was fitted to an exponential profile $n(x) = n_0 \exp(-x/L)$ (Supplementary Information, Fig. S4). The average magnitude of the fluid flow can be estimated from the average length scale $\langle 1/L \rangle = 0.10 \pm 0.02 \mu\text{m}^{-1}$ (mean $\pm$ s.e.m., $n = 30$ cells) obtained from this fit, together with the average apparent diffusion coefficient, $\langle D \rangle = 1.15 \pm 0.1 \mu\text{m}^2 \text{s}^{-1}$ (mean $\pm$ s.e.m. at $t = 0.3$ s, $n = 37$ cells), giving $V_f \sim D/L \sim 0.11 \mu\text{m s}^{-1}$. Thus, in the cell frame of reference we observed fluid flow towards the leading edge with a magnitude of about 40% of cell speed ($\langle V_{\text{cell}} \rangle = 0.27 \mu\text{m s}^{-1}$, $n = 30$ cells).

To understand the origin of this flow, we developed a physical model describing the behaviour of the cytosolic fluid in a moving lamellipodium (Fig. 3a). In the moving cell frame of reference, let $\vec{V}_a$ be the actin network retrograde flow rate. The relative velocity between the actin meshwork and the fluid phase is proportional to the pressure gradient, according to the Darcy flow equations^{1,3}: $\vec{V}_f - \vec{V}_a = -K \nabla P$, where $\vec{V}_f$ is the fluid velocity, P is the hydrostatic pressure, and K is the cytoskeletal permeability. As the actin meshwork in keratocytes is nearly stationary relative to the substrate^{20,21}, we assumed that $\vec{V}_a$ is constant; its magnitude is equal to cell speed and it is directed rearward. The perpendicular centripetal actin network flow at the cell rear^{20–22} did not have a significant effect on fluid flow in the lamellipodium (Supplementary Information, Fig. S5) and was therefore disregarded.

The permeability of the lamellipodial membrane is concentrated at the leading edge^{4,5}, so fluid transport across the membrane will be concentrated there. At the front boundary, the fluid outflux is proportional to the pressure drop across the membrane:

$\vec{V}_f \cdot \vec{n}|_{\text{front}} = k_m (P - P_{\text{out}})|_{\text{front}}$, where $\vec{n}$ is a unit vector locally normal to the front boundary, and k_m is the membrane permeability^{1,23}. At the rear of the lamellipodium, we assumed a constant pressure, $P = P_r = \text{const}$. Together with the fluid incompressibility condition

$\nabla \cdot \vec{V}_f = 0$, we obtained the following equations for the pressure-flow model:

$$\vec{V}_f - \vec{V}_a = -K \nabla P; \nabla \cdot \vec{V}_f = 0; \vec{V}_f \cdot \vec{n}|_{\text{front}} = k_m (P - P_{\text{out}})|_{\text{front}}; P|_{\text{rear}} = P_r \quad (1)$$

Solving this model analytically in 1D (Supplementary Information, Supplementary Text A1) gives an expression for the fluid flow speed V_f as a function of the parameters P_{out} , P_r , V_a , k_m and k_c :

$$V_f = \frac{k_c k_m}{k_c + k_m} (P_r - P_{out}) - \frac{k_m}{k_c + k_m} V_a \quad (2)$$

where $k_c = K/L_0$ is the hydraulic permeability¹ of the meshwork and L_0 is the lamellipodial length. Under normal conditions, the pressure at the rear of the lamellipodium (P_r) is large enough to sustain forward-directed fluid flow and overcome the drag of the actin meshwork (the second term in (2)). This simplified 1D solution neglects the fluid flow perpendicular to the direction of cell movement, yet it gives the correct order of magnitude for the average forward flow, and because it is solvable analytically, provides simple insight and estimates.

The full 2D model was solved numerically (Fig. 3c–e; Supplementary Information, Supplementary Text A2). The hydrostatic pressure drops from rear to front and sides (Fig. 3d) and drives a fountain-like fluid flow against the drag of the actin cytoskeleton (Fig. 3e). Using the measured diffusion rates (Fig. 1), the model predicts the size-dependent probe distribution in 2D (Fig. 3c), which can be compared directly to our experimental results (Fig. 3b). The model-based distribution nicely captured the observed enhancement of the large probe near the leading edge, as well as its accumulation at the lamellipodial wings.

We hypothesized that the increased pressure at the rear of the lamellipodium results from myosin activity. Myosin II is known to localize towards the rear of the lamellipodium in keratocytes^{11,24} and contract the actin meshwork. This contraction creates a compressive stress on the actomyosin network, which can lead to an increase in the local hydrostatic pressure¹. Inhibition of myosin II would therefore be expected to lead to a reduction in the hydrostatic pressure at the rear of the lamellipodium and, thus, according to the model (equation (2), with $P_r - P_{out} \sim 0$), to a reversal in the direction of fluid flow. To test the role of myosin in generating the forward-directed fluid flow, we inhibited myosin II activity with blebbistatin²⁵ and measured the effect on probe distribution. We found depletion of the large probe near the leading edge in blebbistatin-treated cells (Fig. 4a, b; Supplementary Information, Fig. S2). The average length scale extracted from the probe distributions was $\langle 1/L \rangle = -0.15 \pm 0.04 \mu\text{m}^{-1}$ (mean $\pm$ s.e.m., $n = 25$ cells), and the average cell speed was $\langle V_{cell} \rangle = 0.17 \mu\text{m s}^{-1}$. The difference between untreated cells in which we found enhancement of the large probe near the leading edge (i.e. $1/L > 0$) and blebbistatin-treated cells in which the large probe was depleted from the leading edge (i.e. $1/L < 0$) was statistically significant ($P < 10^{-4}$, t -test on the distributions of $1/L$; Supplementary Information, Fig. S4).

The full 2D model was solved for a blebbistatin-treated cell by simply changing the boundary condition for the pressure along the rear to $P_r = P_{out}$ (that is, equal to the outside pressure), giving the predicted probe density (Fig. 4c) and fluid velocity field (Fig. 4d) for a blebbistatin-treated cell. The pressure in a blebbistatin-treated cell still increases from front to rear, but only slightly, so the pressure gradient cannot overcome the actin meshwork drag, and the overall fluid flow is rearward in the cell frame of reference (Fig. 4d). Again, we found good agreement between the measured probe distributions (Fig. 4a), and the model-predicted probe density (Fig. 4c) in the lamellipodium.

Staining of filamentous actin showed that blebbistatin did not alter the morphology of the actin network near the leading edge (Fig. 4e). Moreover, single-particle tracking experiments in live blebbistatin-treated cells did not reveal any substantial differences with respect to QD655 behaviour (Fig. 1e). Furthermore, recent work²¹ has shown that blebbistatin treatment does

not change the characteristics of the actin network flow in the front lamellipodium. These observations further support the notion that the differences in QD distribution seen in blebbistatin-treated cells arise from changes in fluid dynamics, rather than alteration of the actin meshwork.

The contribution of myosin-dependent forward-directed fluid flow to the motility process has been postulated for a long time^{2,8,26}. In motile keratocytes, we found a forward-driven fluid flow caused by myosin activity, with a magnitude of about 40% of cell speed ($\sim 0.11 \mu\text{m s}^{-1}$) in the cell frame of reference. Theoretical estimates (Supplementary Information, Supplementary Text A5, A10) suggest that this forward-directed fluid flow expedites actin monomer transport to the leading edge, and slightly increases the hydrostatic pressure there, effects which could account for a significant ($\sim 20\%$) increase in cell speed in untreated cells, compared with myosin-inhibited cells. These estimates are consistent with our observations that movement of blebbistatin-treated cells is about 40% slower than untreated cells; we hypothesize that about half of this effect is directly related to the myosin-driven forward-directed fluid flow, whereas the other half is related to the role of myosin in reducing membrane tension and in increasing the actin monomer concentrations by promoting actin disassembly at the rear^{20,21}. Taken together, these results indicate that forward-directed fluid flow is not essential for cell motility, yet flow can accelerate the motility process. Finally, any forward (rearward) directed fluid flow in the lamellipodium generates an outflux (influx) at the leading edge that must be balanced by a corresponding influx (outflux) at the cell rear. As discussed in the Supplementary Information, actin–myosin contraction can draw fluid from the surroundings into the cell body, from where fluid can then flow into the lamellipodium. As the surface area at the rear of the cell is an order of magnitude larger than that at the leading edge, the associated fluid flow at the rear would be undetectable.

The existence of a myosin-generated pressure gradient has been documented in other motile cells²⁶. Furthermore, observations in fibroblasts point to the existence of myosin-dependent rapid transport of actin towards protruding regions at the leading edge⁸, which was hypothesized to be at least partially driven by fluid flow towards the edge. The direction of rapid actin transport observed⁸ is consistent with our results in keratocytes, whereas the magnitude of active transport inferred (up to $5 \mu\text{m s}^{-1}$) is substantially higher than our results in keratocytes ($\sim 0.1 \mu\text{m s}^{-1}$). This difference might be caused by actin-specific myosin-dependent active transport mechanisms, in addition to fluid flow, or alternatively may reflect differences in the dynamics of the motility process in these substantially slower-moving cells. The relative contribution of fluid flow to motility will depend on the particular dynamics of the system and on the balance between pressure-mediated and assembly-mediated protrusion. These characteristics will vary among cell types, and even among individual cells within a single cell type¹⁷ or individual cells in a changing environment (Supplementary Information, Fig. S6). In cases where membrane permeability at the leading edge is low, theory predicts that the pressure at the leading edge would increase and this could significantly assist other force generators there. Alternatively, high membrane permeability at the leading edge, as in keratocytes, relieves this pressure but leads to an increased forward-directed fluid flow which accelerates transport to the leading edge. Importantly our work suggests that in both cases the effect of myosin-generated contractile pressure at the rear is beneficial for motility^{4,7}.

Although improving technologies have enabled detailed observation of live keratocytes undergoing rapid actin-based motility, and measurement of traction^{27,28} and protrusive forces²⁹ as well as the dynamic behaviour of specific elements of the motile machinery including actin^{20,22}, myosin¹¹, components of the membrane¹³ and adhesive contacts³⁰, the fluid remained an invisible part of the puzzle. There have been suggestions in the literature for decades² asserting the importance of the fluid in cell motility, but experimental observations have been lacking. Here we show for the first time that fluid dynamics in moving cells can

actually be measured, and this opens the way towards understanding the role of fluid flow in actin-based cell motility.□

METHODS

Methods and any associated references are available in the online version of the paper at <http://www.nature.com/naturecellbiology/>

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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METHODS

Cell culture and sample preparation

Keratocytes were isolated from the scales of the central American cichlid *Hypsophrys nicaraguensis* and cultured as described previously³¹. Briefly, cells were cultured in Leibovitz's L-15 medium (Gibco BRL, Invitrogen) supplemented with 14.2 mM HEPES pH 7.4, 10% fetal bovine serum (FBS) and 1% antibiotic-antimycotic (Gibco BRL), and used 1 day after isolation. 5K methoxypolyethyleneglycol 655QDs or 565QDs (Qtracker), fluorescein-conjugated 500K dextran, Texas red-conjugated 3K dextran, AlexaFluor488 free dye, AlexaFluor594 free dye (all from Molecular Probes, Invitrogen), were introduced into live keratocytes using a small volume electroporator for adherent cells³¹. Cells were placed in a 35 mm dish in 1 ml of culture medium, and the fluorescent probe in 20 µl water was placed directly onto the cell sample. 655QDs or 565QDs were used at 2 µM for distribution measurements and at about 2 nM for single-particle tracking. Following electroporation, cells were allowed to recover in culture medium for at least 10 min. To obtain single isolated cells, sheets of keratocytes were disaggregated by incubation for 5 min in 85% PBS/2.5 mM EGTA pH 7.4 and then returned to culture medium. For visualizing the actin meshwork, cells were fixed in 4% formaldehyde and stained with TRITC-phalloidin (Molecular Probes). Blebbistatin (active enantiomer, Toronto Research Chemicals) treatment was performed at a final concentration of 50 µM blebbistatin in normal medium. Cells were imaged 10–60 min after treatment. Because of the phototoxic effects of blebbistatin on illumination with wavelength <500 nm³², all pre-acquisition adjustments and focusing in the confocal ratio imaging were performed with longer wavelength illumination. No significant effects on cell morphology or speed were observed >60 s after image acquisition. All single-particle imaging was performed using longer wavelengths.

Single-particle imaging

Single-particle imaging of 655QDs in motile keratocytes (~1–20 QDs/cell) was performed in a live-cell chamber at room temperature on either one of two inverted microscopes. In the first set up, cells were imaged using a $\times 60$ (NA = 1.45) objective (1 pixel = 0.11 μm). Movies of 500–1000 frames were collected on an EMCCD (Andor IXON; Andor technology) using either an exposure and frame interval of 0.015 s or an exposure of 0.008 s and a frame interval of 0.038 s. Alternatively, cells were imaged on a Nikon Diaphot 300 microscope using a $\times 40$ (NA = 1.3) lens. Images were collected on a cooled back-thinned CCD camera (MicroMax 512BFT; Princeton Instruments), with a $\times 2$ optovar attached (1 pixel = 0.17 μm). Phase-contrast images were acquired before and after acquisition of a fluorescence movie of 200–500 frames in the streaming mode at a frame interval of about 0.15 s, using exposure times of 0.05 s.

Confocal ratio imaging

Ratio imaging of the distribution of small and large probe in live motile keratocytes was performed at room temperature in a Leica SP2 AOBs confocal laser scanning microscope with a $\times 63$ oil lens (NA = 1.4). Two probes, one green (565QDs, fluorescein-conjugated 500kD dextran or AlexaFluor488 free dye) and one red (655QDs, Texas red conjugated 3kD dextran or AlexaFluor594 free dye), were excited and imaged simultaneously using acousto-optical beam splitters that spectrally split the emitted light onto two separate detectors.

Imaging of fixed cells

Images of fixed cells were collected with Zeiss Axioplan 2 using a $\times 63$ oil lens (NA = 1.4). Images were collected on a cooled CCD camera (MicroMax 512BFT; Princeton Instruments), with a $\times 2$ optovar attached.

Single-particle tracking

Single-particle tracking was performed using custom-written code in Matlab version 7.0 and the image analysis toolbox (The MathWorks). A Gaussian filter was applied to the raw fluorescence images and spots were detected by thresholding. Spot positions were calculated as the centre of mass of the thresholded region, with an accuracy of about 1 pixel. This was sufficient because the particle movement during the exposure time was larger. Spots from consecutive images were linked as described previously³³. Tracks were bridged over short QD blinking events (<10 frames). Longer blinking events lead to the genesis of new tracks.

Ratio distribution measurements

Measurements were performed using custom-written code in Matlab version 7.0 and the image analysis toolbox. The background-corrected intensity profiles of a pair of confocal images (red and green probe) acquired simultaneously were averaged along identical 2- μm wide (= 17 pixels) cross-sections defined manually across the central lamellipodium. A linear fit was performed to the log of the ratio between the intensities $\log(n(x)) = \log(n_0) - x/L$ for x in the range of 1–5 μm from the leading edge, and the best-fit value for $1/L$ was extracted for each cell. This value did not depend on the normalization of the intensities. We disregarded cells with obvious distortions in the generally flat lamellipodium, since they contained dense actin structures in which QDs could be trapped thus disrupting our analysis. Such distortions were more common in cells treated with blebbistatin.

Dynamic light scattering

QD size was determined by dynamic light scattering at 20°C at a concentration of about 0.05 μM QDs in water on a DynaPro 801 instrument (Protein Solutions). The diameter of the 655QDs was measured to be 30.5 ± 1 nm, and the diameter of the 565QDs was 23 ± 2 nm.

2D simulations

The system of partial differential equations for the 2D hydrostatic pressure and fluid velocity distributions was analysed as described in the Supplementary Information and solved using the Virtual Cell (<http://vcell.org>) biological modelling framework³⁴. The corresponding 2D model is publicly available in the Virtual Cell.

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Figure 1.

Single-particle tracking of QDs in the lamellipodia of live keratocytes. **(a)** Phase-contrast (top) and fluorescence (middle; white line: cell outline) images of a keratocyte with 655QDs. The QD trajectories during 100 frames ($dt = 0.15$ s) are shown in the cell frame of reference (bottom; dots indicates $t = 0$). Gaps in the tracks caused by QD blinking are depicted as dashed lines. **(b)** $MSD(t) = \langle \delta r(t)^2 \rangle$ (mean $\pm$ s.e.m.) of 655QDs as a function of time lag calculated on non-overlapping time intervals for data pooled from 12 cells, acquired with an exposure of 0.008 s and frame interval of 0.038 s. The MSD was slightly subdiffusive on all the timescales observed ($0.038\text{ s} < t < 10\text{ s}$; Supplementary Information, Supplementary Text B1), with $MSD(t) \sim t^\gamma$ and $\gamma = 0.89 \pm 0.01$. **(c)** Analysis of the distribution of apparent diffusion coefficient, $D = MSD(t)/4t$; $t = 0.038$ s, for the data shown in **b**; $n = 611$ tracks, truncated to 20 time-points, were included in the analysis (individual QD tracks may be split into several tracks because of long blinking events). The line represents the error distribution¹⁶, assuming a homogenous underlying diffusion coefficient equal to the mean of the distribution, $D = 1.07 \mu\text{m}^2 \text{s}^{-1}$. The experimental distribution is substantially broader than the error distribution, reflecting heterogeneity in the surroundings, probably because of variation in the density and organization of the actin cytoskeleton in different regions within the lamellipodium, and more substantially between different cells. **(d)** Examples of the distribution of apparent diffusion coefficient as in **c** for tracks from two different cells; $n = 57$ (cell A) and $n = 105$ (cell B) tracks, truncated to 15 time-points, were included in the analysis. The QDs in the two cells show markedly different diffusion rates. **(e)** MSD of 655QDs as a function of time lag for individual untreated cells (left) and for blebbistatin-treated cells (right). Each line represents average values for a single cell calculated on non-overlapping time intervals. The apparent diffusion varied considerably

among individual cells between about $0.4\text{--}2.5\ \mu\text{m}^2\ \text{s}^{-1}$ with an average of $D = 1.15 \pm 0.5\ \mu\text{m}^2\ \text{s}^{-1}$ (mean $\pm$ s.d., $D = MSD(t)/4t$; $t = 0.3\ \text{s}$; $n = 37$ cells) for untreated cells and $D = 0.9 \pm 0.3\ \mu\text{m}^2\ \text{s}^{-1}$ ($n = 16$ cells) for blebbistatin-treated cells. The apparent diffusion coefficient in cells was approximately an order of magnitude lower than in water ($D = 14 \pm 0.5\ \mu\text{m}^2\ \text{s}^{-1}$, from dynamic light scattering measurements) because of the crowded intracellular environment¹⁹.

Figure 2.

Flow-induced size-dependent distribution of probes. **(a)** The expected distribution of a large probe diffusing with $D = 1 \mu\text{m}^2 \text{s}^{-1}$ as a function of distance from the leading edge for different flow rates along the anterior–posterior axis. **(b)** The expected distribution of a probe as a function of distance from the leading edge at a constant flow rate of $V_f = 0.1 \mu\text{m s}^{-1}$ towards the leading edge for different values of the probe's diffusion coefficient. **(c, d)** Confocal images of probe distribution in live keratocytes. The distribution of small probe (AlexaFluor488 in **c**, Texas red conjugated 3 kD dextran in **d**; upper images), large probe (QD655 in **c**, Fluorescein-conjugated 500K dextran in **d**; centre images) and the ratio between large probe to small probe (lower images) are shown. Plots of the probe density along cross-sections in the central

lamellipodium show enhancement of large probe near the leading edge (lower panel). The density was measured along the lines shown in the ratio images, and averaged over a width of 2 μm (small probe, green line; large probe, red line; ratio, black line). Control experiments measuring the ratio of two small probes (AF488 and AF594) showed a flat profile (Supplementary Information, Fig. S2). Note that the QDs are excluded from the region very close ($<1 \mu\text{m}$) to the leading edge, probably because of size exclusion by the dense brush-like actin network present there¹¹.

Figure 3.

Measurements of fluid flow based on the distribution of 655QDs. **(a)** An illustration of a keratocyte in the cell frame of reference depicting the model parameters and variables. Side (left) and top (right) views are shown. **(b, c)** The density distribution (left panels) and cross-sections along the black anterior–posterior (centre panels) and grey side–side (right panels) lines (averaged over a width of 2 μm) are shown. The experimental results **(b)** are compared with the results of a simulation **(c)** of the steady-state distribution of 30,000 particles ($\sim$ the number of 655QDs in **b**) diffusing with $D = 1 \mu m^2 s^{-1}$ within a fluid flowing with the velocity distribution shown in **e**. Note the fluid-flow induced enhancement of QDs near the leading edge and towards the sides in both the experimental **(b)** and simulated **(c)** distributions. **(d, e)**

The Darcy flow equations described in the text were solved numerically in 2D (see Supplementary Information for details). The resulting pressure distribution (**d**) and fluid flow velocity distribution (**e**) are shown. The hydrostatic pressure drops from rear to front and sides and drives a fountain-like flow against the drag of the actin cytoskeleton. The fluid encounters more resistance flowing forwards against the retrograde actin meshwork flow, than flowing towards the sides, hence some of the fluid escapes to the lamellipodial sides. As the fluid is incompressible, this leads to a decrease in the flow rate towards the leading edge.

Figure 4.

Effects of blebbistatin on fluid flow. **(a)** Confocal ratio imaging of a cell treated with blebbistatin. The distribution of small probe (upper image), large probe (centre image) and the ratio between large probe to small probe (lower image) are shown. Plots of the probe density along a cross-section in the central lamellipodium show depletion of large probe near the leading edge (lower panel). The density was measured along the line shown in the ratio image, and averaged over a width of $2\ \mu\text{m}$ (small probe, green line; large probe, red line; ratio, black line). **(b)** The normalized ratio density profiles as a function of distance from the leading edge along a cross-section in the central lamellipodium for different cells were averaged. The bold lines and shaded regions represent the mean and standard deviation (respectively) of the profiles of all cells for untreated (red) and blebbistatin-treated (blue) cells. **(c)** A simulation of the steady-state distribution of 30,000 particles diffusing with $D = 1\ \mu\text{m}^2\ \text{s}^{-1}$ within a fluid flowing with the velocity distribution showed in **d**. Fluid-flow-induced depletion of QDs near the leading edge is visible both in the experimental **(a)** and simulated **(c)** distributions. The comparison between experimental results and model should be limited to the lamellipodium, as the model does not address pressure, fluid flow and probe distribution in the cell body at the rear. **(d)** The Darcy flow equations described in the text were solved numerically in 2D for a blebbistatin-treated cell (with boundary condition that $P_r = P_{out}$; see Supplementary Information for details) and the fluid flow velocity distribution is shown. **(e)** Phase-contrast and fluorescence images of an untreated and a blebbistatin-treated cell fixed and stained with phalloidin to visualize actin filaments. The ordered actin meshwork in the lamellipodium is apparent in both the untreated and treated cell.