Soft Matter

PAPER

View Article Online
View Journal | View Issue

Cite this: *Soft Matter*, 2014, **10**, 3421

Intrinsic fluctuations of cell migration under different cellular densities

Song Chen,[a] Na Li,[a] Su-Fan Hsu,[b] Jiazheng Zhang,[a] Pik-Yin Lai,[c] Chi-Keung Chan[b] and Wei Chen*[a]

Received 31st October 2013
Accepted 12th February 2014

DOI: 10.1039/c3sm52752b

www.rsc.org/softmatter

The motility of the Dictyostelium discoideum (DD) cell is studied by video microscopy when the cells are plated on top of an agar plate at different densities, n. It is found that the fluctuating kinetics of the cells can be divided into two normal directions: the cell's forward-moving direction and its normal direction. Along the forward-moving direction, the slope of the amplitude of fluctuation *vs.* velocity ($R_{||}(v)$) increases with n, while along the normal direction the slope of $R_{\perp}$ is independent of n. Both $R_{||}$ and $R_{\perp}$ are functions of the cell speed v. The observed linearity in $R_{\perp}(v)$ indicated that the amplitude of orientational fluctuation (κ) of DD cells is a constant independent of v. The independence of the slope of $R_{\perp}(v)$ on n indicated that κ is also not affected by cellular interactions. The independence of κ on both v and n suggests that orientational fluctuation originates from the intrinsic property of motion fluctuations in DD.

1 Introduction

It is well-known that cells tend to move towards regions with higher concentration of chemo-attractants (such as cAMP for DD), a phenomenon known as chemotaxis,[1,2] which is essential for their biological function. For observations lasting for 1 to 2 days, the motion of cells appears to be rather perplexing with a strong stochastic component as exemplified by their large local fluctuations in velocity magnitude and direction. Previous experiments show that the common Ornstein–Ulenbeck process cannot describe the experimental cell motility data well,[3–6] except at short time lapses. One can view the motion of a DD cell as a self-propelled random walker, with its own and/or external driving fluctuations. However, unlike thermal fluctuations in Brownian motion, little is known on the nature of the fluctuations in cell motion. One method of statistical analysis is developed to extract the deterministic and stochastic components in experimental data,[7–9,24] and has been applied to study cell motions,[10] cardiac dynamics[11] or rough surfaces.[12] In the classical picture of Brownian motion, these two parts correspond mathematically to the effective viscous dissipations and fluctuations experienced by a micro-sphere in a viscous liquid respectively. The property of these fluctuations of a cell is a fascinating issue, presumably related to cell decision making in intracellular processes and intercellular signaling. A detailed statistical analysis on cell pseudopodia showed that the motility fluctuations of cells include both the *de novo* and split modes.[13] A periodic left-right waddle motion was observed as a common feature of the cellular motion, as found in many studies.[4,7,13,14] The decision-making process of the cells could be enhanced under a suitable noise level (stochastic resonance[15]) or by cell–cell communications in a large population such as quorum sensing.[16–18,25] Many efforts were devoted to understand the physics in cell motions, and can account for some of their motion characteristics.[4–6,19–21] In particular, Li *et al.*[7] established a precise and elegant model to describe the dynamics of DD cells and discovered decoupled fluctuations in the motion of the cells, which hence do not need anomalous diffusion interpretation.[7] However, how these decoupled fluctuations will respond to cellular interactions or the cell's internal status are still lacking a good understanding.

In this paper, we report experimental studies on the dependence of velocity fluctuations of DD cells on the cell plating density n. We find that the amplitude of fluctuations in the tangential or forward-moving direction, $R_{||}$, is not only affected by the cell's internal status such as instantaneous speed v, but is also strongly influenced by external environmental factors such as cell plating density n or plating medium (in air or PBS). On the contrary, the fluctuations along the normal direction $R_{\perp}$ do not change much with either n or the plating medium. Furthermore, the linearity of $R_{\perp}$ *vs.* v implies that the angular orientational fluctuation amplitude is a constant independent of the cell's speed, which serves as a key signature for the intrinsic fluctuations in cell motion.

The remainder of the paper is organized as follows. We first describe the experimental procedures and data processing

[a]*Department of Physics, Fudan University, Shanghai 200433, China. E-mail: phchenwei@fudan.edu.cn*

[b]*Academia Sinica, NanKang, Taipei, Taiwan 115*

[c]*Department of Physics and Graduate Institute of Biophysics, National Central University, Chungli, Taiwan 320*

method in the second section. The experimental results and discussion are given in the third section. Finally, the work is summarized in the last section.

2 Experimental

Two cell types, NC4 and KAx-3 of Dictyostelium discoideum (DD), are used in our experiments and are obtained by the following procedures. For NC4 cells, cells of *E. coli* and NC4 are mixed and grown on top of an agar plate with nutrition (15 g agar, 10 g D-glucose, 10 g peptone, 1.44 KH_2PO_4, and 0.38 g Na_2HPO_4 per 1 L distilled water) for the *E. coli*. The mixture of cells is grown for about 36 hours in an incubator kept at 23 °C. Then the NC4 DD cells are isolated from the *E. coli* by centrifuging at 100 *g* and re-suspended in Bonner Salt Solution (BSS). For the KAx-3 cells, they are plated on top of a solid medium with nutrition (20 g agar, 10 g glucose, 10 g tryptone, 1 g yeast extract, 1.9 g KH_2PO_4, 0.78 g $K_2HPO_4 \cdot 3H_2O$ and 1 g $MgSO_4$ per 1 L distilled water), grown in an incubator kept at 23 °C for about 36 hours, and then isolated by centrifuging at 100 *g* and re-suspended in Phosphate Buffer Solution (PBS). The isolated cells of NC4 or KAx-3 are respectively starved for 4 hours in a refrigerator kept at 4 °C before they are plated with the desired density on top of a 1.5% agar plate (without any nutrition). The ages of the gels used in the experiments are between 3 and 14 days. All the procedures mentioned above are carried out under a clean hood. To eliminate uncertainties with different cultures, the experiments under different conditions (such as cell plating density or environment) are conducted using the cell culture obtained at the same time. During the whole experimental observation procedure, the environmental temperature is controlled at 25 °C within ±1 °C. Illumination of the sample by the microscope is on all the time during the experiment. An objective with 4× magnification is used since as many cells as possible need to be recorded to achieve good statistics. Microscope images of the migrating cells are digitized and stored in a computer equipped with a CCD (Prosilica E680, spatial resolution 640 × 480). The time interval between images is 40 seconds due to the pixel limit and the slow speed of the DD cells. The centroid of a cell is recognized by averaging the pixel positions and tracked by homemade software, then the trajectories of the cells are obtained.

Cell displacement $\vec{s}$, velocities $\vec{v}$, mean speed v and turning angle θ during a time interval Δt can be obtained from the trajectories of the cell, as shown in Fig. 1a. Using an orthogonal coordinate along the cell trajectory, the change in cell velocity, $\Delta\vec{v} \equiv \vec{v}_2 - \vec{v}_1$, can be decomposed into two orthogonal components: $\Delta v_{||} = |v_2|\cos\theta - |v_1|$ and $\Delta v_\perp = |v_2|\sin\theta$, where the subscripts of $||$ and $\perp$ represent the tangential and normal components respectively. To incorporate the stochastic nature and fluctuations of these motion components of the cell, one can describe these components using two general stochastic equations as follows:[22]

$$\frac{\Delta v_\parallel}{\Delta t} = -h_\parallel(v) + R_\parallel(v)\Gamma_\parallel(t), \quad (1)$$

$$\frac{\Delta v_\perp}{\Delta t} = -h_\perp(v) + R_\perp(v)\Gamma_\perp(t), \quad (2)$$

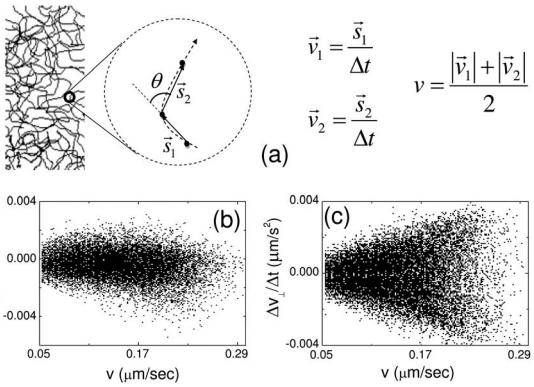

Fig. 1 (a) Definitions of cell displacement vector $\vec{s}$, velocity vectors $\vec{v}$ and instant mean speed v during a time interval Δt. Solid curve represents a trajectory of a cell with the arrow showing the forward-moving direction of the DD. The bullet points on the curve represent the cell locations recorded in a time interval Δt. θ is the turning angle between two successive time intervals. (b) Experimental data of the NC4 cells: (b) $\Delta v_{||}/\Delta t$ *vs.* v, (c) $\Delta v_\perp/\Delta t$ *vs.* v. Cell density $n = 100$ cells per mm^2 and $\Delta t = 40$ s.

where $\Gamma(t)$ is the unit-variance noise with zero mean. $h(v)$ and $R(v)$ represent the "dissipative" terms and noise amplitudes respectively. The velocity in the experiment is always obtained from the difference of the location of the cell at t and $t + \Delta t$, which is in some sense an averaged velocity in a time interval of Δt. Thus eqn (1) and (2) should not be interpreted as stochastic differential equations, but rather as modeling some average deviation of the components of the velocity over some time interval, with the RHS being dependent on the average speed around this time. We choose $v = (|v_1| + |v_2|)/2$ because it can better represent the mean speed of the cell in the time interval. Fig. 1b and c show the data of $\Delta v_{||}/\Delta t$ and $\Delta v_\perp/\Delta t$ *vs.* v for NC4 cells obtained experimentally, which are extracted from the cell trajectories.

3 Experiment results

Similar to the methods used in ref. 10 and 19, an ensemble of averages of eqn (1) and (2) are taken. Assuming a zero-mean noise $\Gamma(t)$, $h(v)$ could be obtained by averaging the data for each mean speed v in Fig. 1b and c. The noise amplitude $R(v)$ can be estimated by

$$R_\alpha^2(v) = \left\langle \frac{(v_\alpha(t+\Delta t) - v_\alpha(t))^2}{2\Delta t} \right\rangle\Bigg|_v, \quad (3)$$

for each given mean velocity v. Here, $\alpha = ||$ or $\perp$ component of the cell. The form of $h(v)$ and $R(v)$ for NC4 cells with different densities are shown in Fig. 2.

The tangential $h_{||}(v)$ follows a linear decrease to a good approximation for most cell densities, suggesting the dissipation coefficient is almost a constant. But for lower cell density, it deviates slightly from a single linearity. On the other hand, the normal $h_\perp(v)$ is very close to zero for all different n, and is independent of the cell speed v or density n. Fig. 2c and d show that both $R_{||}$ and $R_\perp$ increase with v. $R_{||}(v)$ shows a clear

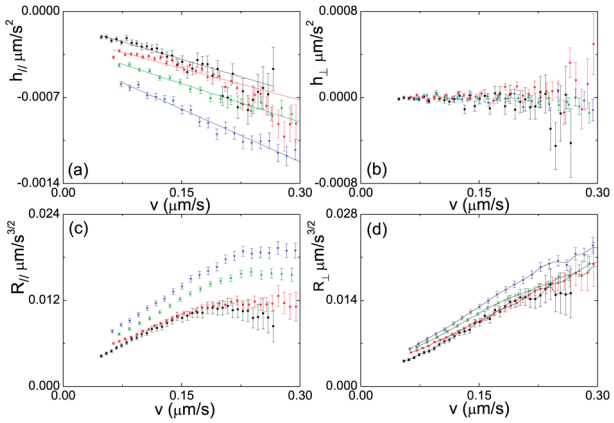

Fig. 2 $h(v)$'s and $R(v)$'s extracted from experimental measurements for NC4 DD cells with different plating density n. (a) $h_{||}$ *vs.* v, (b) $h_{\perp}$ *vs.* v, (c) $R_{||}$ *vs.* v, and (d) $R_{\perp}$ *vs.* v. Symbols: square (black), bullet (red), up-triangle (green) and down-triangle (blue) stand for density of n = 100, 300, 600, 1000 (cells per mm^2) respectively. The solid lines in (a) denote the linear fits.

dependence on n, while the dependence of $R_{\perp}$ on n is much weaker. $R_{||}(v)$ can be fitted with a linear form $R_{||}(v) \approx \mu + \lambda(n)v$ for $v < 0.2$ μm s^{-1}. The value of the intercept μ is a constant close to zero within our experimental error. At the high speed region $v > 0.2$ μm s^{-1}, $R_{||}$ tends to saturate with v, which suggests the relation between $R_{||}(v)$ will be more complicated for cells at high speeds. But the high speed cell sample is less than 10%. The two curves of $R_{||}(v)$ for n = 100, 300 mm^{-2} are almost overlapping, indicating that the amplitude of $R_{||}(v)$ is hardly affected by n when n is small. It is a typical feature of quorum sensing: cells begin to respond to the intercellular communication only when the cell density exceeds some critical level. The most important feature in Fig. 2c and d is that the slopes of $R_{\perp}(v)$ for different n are almost the same, suggesting the approximate form of $R_{\perp}(v) = \kappa(v)$, which implies that fluctuations in the normal component of the velocity change are not affected by intercellular communications.

One can substitute the approximate forms of $h(v)$ and $R(v)$ into eqn (1) and (2). From Fig. 2, one has $h_{||}(v) = -\eta v$, $R_{||}(v) \approx \lambda v$, $h_{\perp}(v) = 0$ and $R_{\perp}(v) = \kappa v$. Eqn (1) and (2) then read:

$$\frac{\Delta v_{||}}{\Delta t} = -\eta v + \lambda v \Gamma_{||}(t), \tag{4}$$

$$\frac{\Delta v_{\perp}}{\Delta t} = \kappa v \Gamma_{\perp}(t). \tag{5}$$

$1/\eta$ represents persistence time. It should be pointed out that the linearity of the multiplicative noise $R(v)$ is valid only when the velocity is below a certain speed, as shown in Fig. 2c. The fitting parameters obtained from Fig. 2 are listed in Table 1. Cell samples with high densities will secrete more chemotactic molecules (cAMP) into their local environment. The results suggest that λ depends on the local environment (cell density), whereas κ is a constant instead.

To verify whether the above unique behavior in cell motion fluctuations is special to the cell type of NC4, we repeat the whole measurement with another typical mutant cell, KAx-3. Three densities n = 100, 240, 500 (cells per mm^2) are prepared for the KAx-3 cells. To verify the effect of intercellular communications on the fluctuations of the cell clearly, the cells are plated in two conditions for each density: on agar gel which is exposed directly to the air (same condition as the NC4 cells above), or on agar gel immersed in PBS. The chemotactic signaling molecules among the cells will diffuse with different rates in air or liquid, thus resulting in different spatial gradient concentration profiles of the signaling molecule, and hence corresponding to different intercellular communication conditions.

The curves of $h_{||}(v)$ and $h_{\perp}(v)$ of KAx-3 cells with different plating conditions are plotted in Fig. 3a and b. The curves of $R_{||}(v)$ and $R_{\perp}(v)$ are plotted in Fig. 3c and d. The curves in Fig. 3a and c show that, for the tangential component, both the dissipation coefficient and the fluctuation of the cell are influenced by external conditions, such as plating density or plating medium (air or PBS). The amplitude of $R_{||}$ from cells immersed in PBS (open symbol) is smaller than that of the cells exposed in air (solid symbol) of the same cell density. However, as shown in Fig. 3d, all curves of $R_{\perp}$ obtained from different plating conditions collapse with each other. This result suggests that the cell environment (intercellular communication indeed) can only affect the noise level of the cell for the velocity change in the tangential direction, but NOT for the normal direction.

From the definition $\Delta v_{\perp} \equiv |v_2|\sin\theta$ and $h_{\perp}(v) = 0$, one gets $R_{\perp}(v)$ = RMSD $(\Delta v_{\perp}/\Delta t) \approx v \times$ RMSD $(\sin\theta)$, where RMSD stands for the root-mean-square deviation. Thus the linear form of $R_{\perp}(v)$ implies that the fluctuation amplitude of the angular orientation, RMSD $(\sin\theta)$, is a constant, which agrees with Shenderov's recent observation of angle distribution of DD cells (Fig. 3c in ref. 4). Eqn (5) can be approximated by an equation for $\sin\theta$:

$$\sin\theta \approx \kappa \Gamma_{\theta}(t)\Delta t. \tag{6}$$

The amplitude of the orientational noise, κ, is independent of the internal status of the cell v, and is also independent of the external condition (plating density). These results suggest that orientational angular fluctuation reflects the intrinsic noise of the DD cell.

It is worth mentioning that the orientational deviation (θ) here corresponds to the noise of split mode rather than the *de novo* mode defined in ref. 13, although it was reported that the *de novo* pseudopodia extended randomly without external cues.[23] Because the time interval of Δt used in our analysis is well below the motion persistent time of the DD cells, most orientational fluctuations are measured during the cell persistent running period and correspond to the split mode: a new pseudopod splits from the existing pseudopodia.[13]

Eqn (4) and (5) show that the fluctuating motion of a cell derived from trajectories of DD cells behaves asymmetrically in two directions. The noise in the normal direction, $R_{\perp}(v)\Gamma_{\perp}$, could be obtained by calculating $\Delta v_{\perp}/\Delta t$ directly since $h_{\perp}(v) \simeq 0$. However, the noise in the tangential (forward-moving) direction, $R_{||}(v)\Gamma_{||}$, has to be obtained by subtracting $h_{||}(v)$ from $\Delta v_{||}/\Delta t$, which in turn will introduce larger errors. On the other hand, if we consider the fluctuation of cell speed by calculating

Table 1 Values of the parameters η, λ, and κ of NC4 cells obtained from fitting for different cell densities n (cells per mm^2) in Fig. 2

n	100 (cells per mm^2)	300 (cells per mm^2)	600 (cells per mm^2)	1000 (cells per mm^2)
$1/\eta$	380 ± 30 (s)	338 ± 40 (s)	357 ± 30 (s)	345 ± 30 (s)
λ	0.049 ± 0.01 ($s^{-1/2}$)	0.05 ± 0.01 ($s^{-1/2}$)	0.063 ± 0.01 ($s^{-1/2}$)	0.073 ± 0.01 ($s^{-1/2}$)
κ	0.067 ± 0.01 ($s^{-1/2}$)	0.067 ± 0.01 ($s^{-1/2}$)	0.067 ± 0.01 ($s^{-1/2}$)	0.067 ± 0.01 ($s^{-1/2}$)

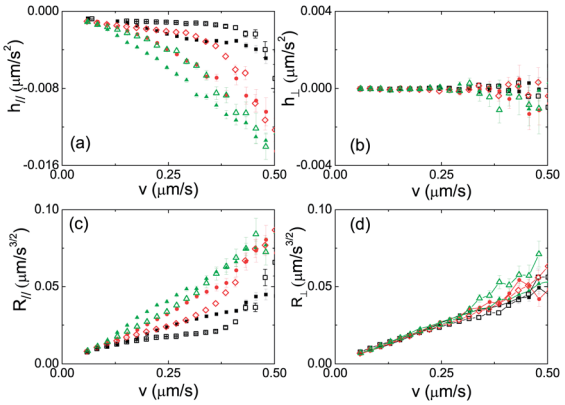

Fig. 3 $h(v)$ and $R(v)$ extracted from experimental measurements for KAx-3 DD cells with different plating density n. (a) $h_{||}$ *vs.* v, (b) $h_{\perp}$ *vs.* v, (c) $R_{||}$ *vs.* v, and (d) $R_{\perp}$ *vs.* v. Symbols: square (black), dot (red), up-triangle (green) represent densities of $n = 100$, 240, 500 (cells per mm^2) respectively. Solid symbols represent the case of cells exposed in air; open symbols represent the case of cells immersed in PBS.

$\Delta v_r/\Delta t \equiv (v_2 - v_1)/\Delta t$ instead, the corresponding generalized stochastic equation of $\Delta v_r/\Delta t$ can be written as:[10]

$$\frac{\Delta v_r}{\Delta t} = h_r(v) + R_r(v)\Gamma_r(t) \quad (7)$$

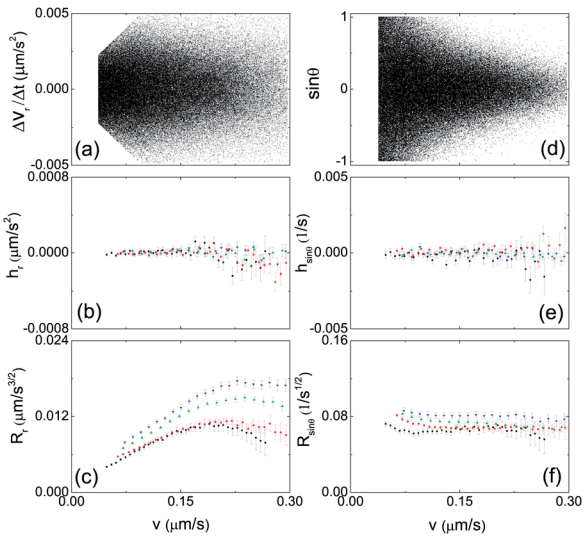

Fig. 4 (a) $\Delta v_r/\Delta t$ *vs.* v for the same NC4 cells as in Fig. 1. (b) The curves of $h_r(v)$ are obtained from (a). (c) $R_r(v)$ obtained from (a). Cell density $n =$ 100 cells per mm^2 and time interval $\Delta t = 40$ s. (d) sin θ *vs.* v. (e) The deterministic part of sin θ obtained by averaging the results in (d). (f) The amplitude of the fluctuating part in (d). Symbols: square (black), dot (red), up-triangle (green) and down-triangle (blue) represent densities of $n = 100$, 300, 600, 1000 (cells per mm^2) respectively.

for some zero-mean and unit-variance white noise Γ_r. Fig. 4a shows the experimental data of $\Delta v_r/\Delta t$ *vs.* v from the same NC4 sample of Fig. 1. From these data, $h_r(v)$ and $R_r(v)$ are calculated using the same method mentioned above. The results are shown in Fig. 4b and c, indicating $h_r(v) \simeq 0$ and the curves of $R_r(v)$ are similar to those of $R_{||}(v)$ in Fig. 2c. The corresponding noise amplitude $R_r(v)$ is also found to be well-approximated by a linear form of $\lambda_r v$. Substituting the above specific forms of $h_r(v)$ and $R_r(v)$, eqn (7) can be rewritten as:

$$\frac{\Delta v_r}{\Delta t} = \lambda_r v \Gamma_r(t). \quad (8)$$

Eqn (6) and (8) together with the two parameters λ, κ can be used to describe the fluctuating motion of DD cells reasonably well. Our model about the velocity fluctuation involves no memory kernel in the stochastic equations, as in ref. 19. Such a memory kernel integral is important to determine the persistent orientation of DD cells,[7] but can be partially compensated by the average in a time interval as long as 40 seconds in this work. The simple forms of eqn (6) and (8) also suggest that the natural coordinate frame is better than the Cartesian frame used in eqn (4) and (5), agreeing with the conclusion in ref. 7. Eqn (6) describes the fluctuations of orientational angle of the cell, which is insensitive to the cell density n or environment. These behaviors are shown in Fig. 4e and f. The strength of orientational fluctuation is almost a constant independent of v. With this in mind, one can envision a cell would not have any preferred direction to move in and its angular orientational noise RMSD(sin θ) would be the only fluctuation left behind. A DD cell still undergoes shape deformations without external stimuli due to the mediation by PI3K/PTEN/F-actin inside.[26] Since RMSD(sin θ) does not change with the intercellular interaction or the cell's own motion status, this noise can be regarded as the intrinsic noise of the cell, analogous to k_BT for a colloidal system.

4 Conclusions

In summary, our experiment demonstrated that the motility fluctuations of cells are decoupled along two directions in the natural coordinates on the trajectory. The orientational fluctuation is independent of both the intercellular interaction or the cell's self-speed, and hence could be regarded as intrinsic fluctuations. The forward-moving fluctuations depend on both the intercellular interaction and cell speed, which can be interpreted as the noise strength being modified by external stimuli or the cell's own status. It will be very interesting to

verify further whether the other motion fluctuations of a cell come from the intrinsic noise, which presumably could be modified by molecular biology means.

Acknowledgements

We thank Prof. LianSheng Hou for the supply of KAx-3 cell samples. This work has been supported by National Science Fund for Talent Training in Basic Science, Grant no. J1103204, NSC of ROC under the grant no.s NSC 100-2923-M-001-008-MY3, 101-2112-M-008-004-MY3, and NCTS of Taiwan.

References

1 B. M. Shaffer, *Nature*, 1953, **171**, 975.
2 A. R. Kimmel and C. A. Parent, *Science*, 2003, **300**, 1525.
3 L. S. Ornstein, *Proc. Amst.*, 1918, **21**, 96; G. E. Uhlenbeck and L. S. Ornstein, *Phys. Rev.*, 1930, **36**, 823.
4 A. D. Shenderov and M. P. Sheetz, *Biophys. J.*, 1997, **72**, 2382.
5 P. Dieterich, R. Klages, R. Preuss and A. Schwab, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 459.
6 H. Takagi, M. J. Sato, T. Yanagida and M. Ueda, *PLoS One*, 2008, **3**, e2648.
7 L. Li, E. C. Cox and H. Flyvbjerg, *Phys. Biol.*, 2011, **8**, 046006.
8 S. Siegert, R. Friedrich and J. Peinke, *Phys. Lett. A*, 1998, **243**, 275.
9 J. Gradisek, S. Siegert, R. Friedrich and I. Grabec, *Phys. Rev. E: Stat. Phys., Plasmas, Fluids, Relat. Interdiscip. Top.*, 2000, **62**, 3146.
10 H. U. Bodeker, C. Beta, T. D. Frank and E. Bodenschatz, *Europhys. Lett.*, 2010, **90**, 28005.
11 T. Kuusela, *Phys. Rev. E: Stat., Nonlinear, Soft Matter Phys.*, 2004, **69**, 031916.
12 G. R. Jafari, S. M. Fazeli, F. Ghasemi, S. M. V. Allaei, M. R. R. Tabar, A. I. Zadand and G. Kavei, *Phys. Rev. Lett.*, 2003, **91**, 226101.
13 P. J. M. V. Haastert, *J. Cell Sci.*, 2010, **123**, 3031.
14 T. D. Yang, J. S. Park, Y. Choi, W. Choi, T. W. Ko and K. J. Lee, *PLoS One*, 2011, **6**, e20255.
15 P. H. L. Gammaitoni, P. Jung and F. Marcheson, *Rev. Mod. Phys.*, 1998, **70**, 1.
16 A. Koseska, A. Zaikin, J. Kurths and J. Garcia-Ojalvo, *PLoS One*, 2009, **4**, e4872.
17 T. Greor, *et al.*, *Science*, 2010, **328**, 1021.
18 W. Y. Chiang, Y. X. Li and P. Y. Lai, *Phys. Rev. E: Stat., Nonlinear, Soft Matter Phys.*, 2011, **84**, 041921.
19 D. Selmeczi, S. Mosler, P. H. Hagedorn, N. B. Larsen and H. Flyvbjerg, *Biophys. J.*, 2005, **89**, 912.
20 V. Zaburdaev, S. Uppaluri, T. Pfohl, M. Engstler, R. Friedrich and H. Stark, *Phys. Rev. Lett.*, 2011, **106**, 208103.
21 J. S. Gruver, A. A. Potdar, J. Jeon, J. Sai, B. Anderson, D. Webb, A. Richmond, V. Quaranta, P. T. Cummings and C. Y. Chung, *Biophys. J.*, 2010, **99**, 367.
22 H. Risken, *The FokkerPlanck-Equation. Methods of Solution and Applications*, Springer, Berlin, 2nd edn, 1996.
23 L. Bosgraaf and P. J. M. Van Haastert, *PLoS One*, 2009, **4**, e5253.
24 H. U. Bodeker, M. C. Rottger, A. W. Liehr, T. D. Frank, R. Friedrich and H.-G. Purwins, *Phys. Rev. E: Stat., Nonlinear, Soft Matter Phys.*, 2003, **67**, 056220.
25 S. Park, P. M. Wolanin, E. A. Yuzbashyan, P. Silberzan, J. B. Stock and R. H. Austin, *Science*, 2003, **301**, 188.
26 Y. T. Maeda, J. Inose, M. Y. Matsuo, S. Iwaya and M. Sano, *PLoS One*, 2008, **3**, e3734.