



**HAL**  
open science

## Probing active forces via a fluctuation–dissipation relation

Pierre Bohec, François Gallet, Christian Maes, Soghra Safaverdi, Paolo Visco,  
Frédéric van Wijland

► **To cite this version:**

Pierre Bohec, François Gallet, Christian Maes, Soghra Safaverdi, Paolo Visco, et al.. Probing active forces via a fluctuation–dissipation relation. 2012. hal-00679065v1

**HAL Id: hal-00679065**

**<https://hal.science/hal-00679065v1>**

Preprint submitted on 14 Mar 2012 (v1), last revised 24 Jun 2013 (v2)

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# Probing active forces via a fluctuation–dissipation relation

P. Bohec,<sup>1</sup> F. Gallet,<sup>1</sup> C. Maes,<sup>2</sup> S. Safaverdi,<sup>2</sup> P. Visco,<sup>1,\*</sup> and F. van Wijland<sup>1</sup>

<sup>1</sup>*Laboratoire Matière et Systèmes Complexes, CNRS UMR 7057, Université Paris Diderot, 10 rue Alice Domon et Léonie Duquet, 75205 Paris cedex 13, France*

<sup>2</sup>*Institute for Theoretical Physics, KU Leuven, Belgium*

(Dated: March 14, 2012)

We derive a new fluctuation–dissipation relation for non–equilibrium systems with long term memory. We show how this relation allows one to access new experimental information regarding active forces in living cells that cannot otherwise be accessed. For a silica bead attached to the wall of a living cell, we identify a cross-over time between thermally controlled fluctuations and those produced by the active forces. We show that the probe position is eventually slaved to the underlying random drive produced by the so-called active forces.

PACS numbers: 05.40.Ca, 87.18.Tt, 87.16.Nn

Living cells are paradigmatic out of equilibrium systems, subjected to the ATP-driven activity of a collection of molecular motors, whose individual motion cannot easily be disentangled from thermal fluctuations. These are relatively small systems for which fluctuation phenomena are prominent, as investigated in recent works [1–4], with specific focus on the active forces spectrum [1, 5] which can be measured via micro-rheological devices. Our goal in the present work is to show how the cell tissue’s random pull-and-push can be investigated by means of very recent theoretical advances in the field of non–equilibrium statistical mechanics. And indeed, from the theory standpoint, in the recent past, much effort has been invested in deriving simple generalizations or extensions of the celebrated fluctuation–dissipation theorem for systems that can be arbitrarily far out of equilibrium [3, 6–13]. These efforts have given birth to a flurry of formulas relating the response of a system to a small external perturbation to some correlation functions, even when the system is not in an equilibrium state. Yet, so far none of these formulas has been of any predictive power in an actual experimental system. Existing experiments revolving around these theoretical advances have been confined to refined and nontrivial confirmations that in some small scale systems such as an optically trapped Brownian particle [14–16] the various ingredients entering these extended fluctuation-dissipation relations (EFDR) can indeed be measured. Our purpose in this work is to exploit an EFDR to access relevant pieces of information on active forces in living cells that cannot otherwise be accessed. Given that the dynamics of living cells exhibit strong memory effects, we will first have to derive our own version of an EFDR adapted to a system with stochastic yet non-Markovian dynamics. The latter EFDR and its consequence for the understanding of active forces are the central topics of this letter. For instance, how relevant is the long term memory in relating response and fluctuations out of equilibrium (the answer is fully negative in equilibrium)? What new pieces of information on non–equilibrium forces can be learnt from such a relationship? Can thermal fluctuations be disentangled from those arising from the active forces in a quantitative way? These are the questions we wish to address in the present work.

Our experimental setup consists of a pre–muscular (C2C12)

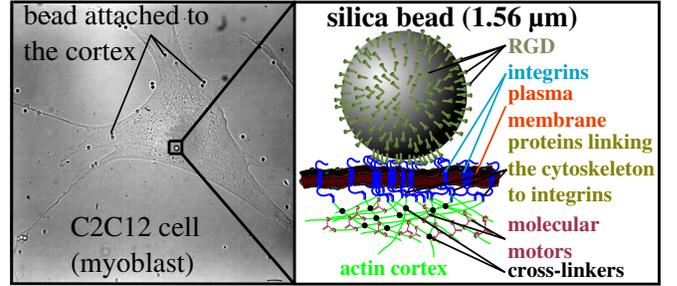


FIG. 1. Schematic view of the bead probe as attached to the cytoskeleton. The active force are the by-product of the ATP-driven molecular motors activity.

cell to which we slave a micron sized silica probe specifically bound to the membrane. Its dynamics strongly feels the viscoelastic non-Newtonian underlying medium [1], which gives rise to memory (non-Markovian) effects. A schematic view of our experimental setup is shown in FIG. 1. We assume that the bead dynamics is well described by a visco–elastic description. Our bead attached to the cell cortex thus evolves under the combined effect of thermal fluctuations  $\xi(t)$  and active forces  $F_a(t)$ . Discarding inertial effects, the position  $x$  of the bead is governed by the Langevin equation

$$m \int_{-\infty}^t dt' \gamma(t-t') \frac{dx(t')}{dt'} = F_a(t) + \xi(t), \quad (1)$$

where the Gaussian colored noise  $\xi$  has correlations  $\sigma(\tau) = \langle \xi(t)\xi(t+\tau) \rangle$ . We assume that the causal function  $\gamma$  is related to  $\sigma$  through a local detailed balance condition [17]:  $\sigma(\tau) = m/\beta\gamma(|\tau|)$ , where  $\beta = 1/k_B T$  is the inverse temperature. The active forces are modeled by an isotropic stationary random force  $F_a(t)$ . While the presence of the memory kernel  $\gamma(\tau)$  looks like an innocuous extension of the well-known overdamped Langevin equation (for which  $\gamma(\tau)$  is to be replaced by a Dirac delta in time), we shall soon see that a number of complications emerge.

We begin with deriving our central theoretical result, namely a relationship between three quantities, two of which

are accessible to measurement. Let  $R(t)$  be the response of the bead position at time  $t$  when applying an external space independent force at time 0. Let also  $C(t) = \langle x(t)x(0) \rangle$  be the auto-correlation function of the bead's position. Our goal is to connect  $R$  and  $C$  to the statistics of the active force  $F_a$ . Then, we shall show how to exploit our mathematical identity to extract useful pieces of information on active forces. Starting from (1), the probability weight for a given realization of the noise is written as  $P[\xi] = \exp(-S[\xi])$ , where the action functional  $S$  reads:

$$S[\xi] = \frac{1}{2} \int dt_1 \int dt_2 \xi(t_1) \sigma^{-1}(t_1 - t_2) \xi(t_2). \quad (2)$$

The function  $\sigma^{-1}$  is the deconvolution inverse of the noise covariance:  $\int dt \sigma(t) \sigma^{-1}(\tau - t) = \delta(\tau)$ . The next step is to rewrite (2) by replacing the noise  $\xi(t)$  with its explicit expression which solves (1). Then, by considering the effect of a small force  $f(t)$  applied to the system, one has a variation from  $S$  to  $S + \delta S$ , where  $\delta S$  is, up to linear order in  $f$ :

$$\begin{aligned} \delta S = & \int dt_1 \int dt_2 \int_{-\infty}^{t_1} dt'_1 [-\beta \sigma(t_1 - t'_1) v(t'_1) \sigma^{-1}(t_1 - t_2) \\ & \times f(t_2)] + [F_a(t_1) \sigma^{-1}(t_1 - t_2) f(t_2)] + \mathcal{O}(f^2). \end{aligned} \quad (3)$$

By defining the response  $R$  to the perturbation  $f$  as  $\delta \langle x \rangle(t) = \int dt' R(t - t') f(t')$  one finds:

$$\begin{aligned} R(t - t') = & \frac{\delta \langle x(t) \rangle}{\delta f(t')} = - \left\langle x(t) \frac{\delta S}{\delta f(t')} \right\rangle \\ = & \beta \int ds \int_{-\infty}^s dr \sigma(s - r) \sigma^{-1}(s - t') \frac{d}{dr} \langle x(r) x(t) \rangle \\ & - \int ds \sigma^{-1}(s - t') \langle F_a(s) x(t) \rangle. \end{aligned} \quad (4)$$

Formula (4) neither simplifies in the stationary (non-equilibrium) limit nor in the absence of active forces  $F_a$  because of the memory-effects. In the Markov limit, the memory kernel is a Dirac delta  $\gamma(t) = \gamma \delta(t)$ , which leads to

$$R(t - t') = \frac{\beta}{2} \frac{d}{dt'} \langle x(t') x(t) \rangle - \frac{\beta}{m\gamma} \langle F_a(t') x(t) \rangle, \quad (5)$$

a formula that as such first appeared in [12] and for which the second term in the right hand side has an interpretation in terms of dynamical activity. The formula being valid for arbitrary forces  $F_a$ , it would also be valid if  $F_a$  were an equilibrium force field, and in that case the terms in the right hand side of (5) add up and yield the standard form of the fluctuation-dissipation theorem. Finally, in the absence of an external force field  $F_a$ , no stationary regime can be reached, but the formula  $R(t - t') = \frac{\beta}{2} \frac{d}{dt'} \langle x(t') x(t) \rangle$  suggests an effective temperature twice that of the bath governs the dynamics, a simple interpretation already noted in [18].

The gist of equation (4) is that both  $R(t - t')$  and the companion correlation function  $\langle x(t') x(t) \rangle$  can independently be

measured, and thus, provided the functions  $\sigma^{-1}$ ,  $\sigma$ ,  $\gamma$  encoding memory effects are well-known, access is granted to the projection of the active force onto the beads position,  $\langle F_a(t') x(t) \rangle$ . Our only requirement is thus to be able to determine the functions  $\gamma$  and  $\sigma^{-1}$ . This can explicitly be carried out by computing the effect of the external perturbation  $\delta \langle x \rangle(t)$  from (1). For this it is useful first to define the inverse memory kernel  $\gamma^{-1}$  such that  $\int \gamma(t) \gamma^{-1}(t - \tau) dt = \delta(\tau)$ . With this we can explicitly solve (1) for  $x(t)$  obtaining:

$$R(t) = \frac{1}{m} \int_{-\infty}^t d\tau \gamma^{-1}(\tau). \quad (6)$$

Before precisely showing how to extract useful information from experimental data, we shall first consider the time dependence of our correlation functions. Since our system is not in a steady state, all correlation functions *a priori* depend on two times. However, since the velocity  $dx(t)/dt$  is in a steady-state, the mean-square-displacement  $\Delta x^2(t, t') = \langle [x(t) - x(t')]^2 \rangle$  only depends on  $t - t'$ . We can then rewrite:

$$\frac{d}{dt'} \langle x(t') x(t) \rangle = \frac{1}{2} \frac{d}{dt'} \langle x^2(t') \rangle + \frac{1}{2} \frac{d}{dt} \Delta x^2(t - t'). \quad (7)$$

Furthermore, the response function only depends on the time difference  $t - t'$ , so that the correlation function  $\langle F_a(t') x(t) \rangle$  can also be decomposed as:

$$\langle F_a(t') x(t) \rangle = \langle F_a(t') x(t') \rangle + C_{xF}(t - t'). \quad (8)$$

We can now deduce the active-force-position correlations from (4), which we can rewrite as a first relation for the initial time  $t'$  and a second one for the time difference  $\tau = t - t'$ :

$$\langle F_a(t') x(t') \rangle = \frac{\beta}{2} \int_{-\infty}^{t'} dr \sigma(t' - r) \frac{d}{dr} \langle x^2(r) \rangle, \quad (9)$$

$$C_{xF}(\tau) = \frac{\beta}{2} \int_{-\infty}^{\tau} dt \sigma(t - \tau) \frac{d}{dt} \Delta x^2(t) - \int dt R(t) \sigma(\tau - t). \quad (10)$$

These formulae allow us to fully determine the time behavior of the correlation function  $\langle F_a(t') x(t) \rangle$ . Of course the physically relevant component of this correlation function is the time-translational invariant one, denoted above as  $C_{xF}$ . In fact the first term in the rhs of (10) only expresses the dependence on the choice of the initial time  $t'$ . Here we shall restrict ourselves to the case where  $t' = 0$  (the general case  $t' \neq 0$  will be discussed elsewhere [19]). Furthermore, the second term in the rhs of (10) corresponds to the correlation function between the position and the thermal noise.

We shall now apply our result to probe active forces in living cells. The experimental set-up is the same as exposed in [4] and consists of a small bead attached to the cortex of a muscular cell (see FIG.1) at room temperature ( $\sim 298\text{K}$ ).

First, we track the free diffusion of the bead on the cell cortex. This allow us to measure the mean square displacement of the probe as a function of time  $\langle \Delta x^2(t) \rangle$ , shown in

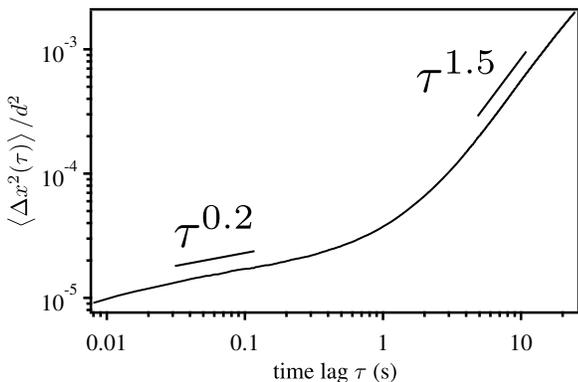


FIG. 2. The probe mean square displacement as a function of time when there is no external applied force. A definite crossover time is found at  $\tau \simeq 1$  s.

FIG. 2. It exhibits a two-step growth characterized by an early sub-diffusion, up to a 1 s. timescale yet to be interpreted, followed by super-diffusion. Second, the response function is measured by applying optical tweezers to the bead. The tweezer acts on the bead as a harmonic potential of spring constant  $k_{opt} = 120\text{pN}/\mu\text{m}$ . After switching our tweezers on we quickly translate them by  $x_0 = 0.6\mu\text{m}$  at time  $t = 0$ , creating a displacement of the bead attached to the cell. In the experiment the external force we apply is hence well approximated by  $f_{exp}[x(t), t] = -k_{opt}(x(t) - x_0)\theta(t)$ , assuming that the displacement at  $t = 0$  is instantaneous. The distance between the bead and the center of the tweezers allows us to measure the force applied on the bead. By repeating this experiment we can measure the average displacement and the average force as shown in FIG. 3. The force suddenly increases at a time of about 0.004s and then slowly decreases towards 0, while the bead displacement increases following a power law behavior (except in the initial steps of the relaxation), as shown in the Log-Log scale inset. This is compatible with the visco-elastic rheology responsible for the subdiffusivity of the mean square displacement [20–22]. The analysis also shows that a sum of two power laws fits extremely well the data over all the experimentally accessible range. Equation (1) implies that  $\langle x \rangle$  and  $\langle f_{exp}[x] \rangle$  are related according to  $\delta \langle x(t) \rangle = \int dt' R(t - t') \langle f_{exp}[x(t'), t'] \rangle$ , where  $R$  is the response to a space independent force, as obtained in (4).

All our experimental data are discretized in time. We first measure trajectories of the bead position when no external force is applied. We have collected  $N_S^0 = 39$  independent sample trajectories of 250s each with a time step of 0.004s (each trajectory consists of  $N_p^0 = 62500$  points). This yields a set of vectors  $x_i^j$ , where the subscript  $i$  stands for the time position and superscript  $j$  refers to the trajectory number. We compute the mean square displacement as  $\langle \Delta x^2 \rangle_i = \sum_{j,k} (x_j^k - x_{j+i}^k)^2 / N_S^0 (N_p^0 - i)$ , from which we take the discrete derivative. A second set of measurements is taken when we apply the external force on the bead. Then

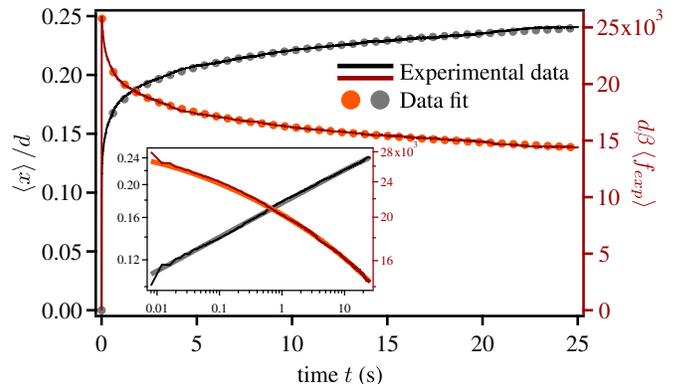


FIG. 3. Average particle position (black) and applied force (red) in dimensionless units ( $d$  is the particle diameter), as a function of time. Data is compared with the best fit used in deconvolving operations to restrain noise amplification.

we measure both the position  $x(t)$  and the external force  $f(t)$ , as shown in FIG. 3. All trajectories are measured for a time window of 25s with a time step of 0.004s and ensemble averages are taken over 39 realizations. We obtain the discrete response function  $R_i$  by deconvolving the discrete external force  $\langle f \rangle$  with  $\delta \langle x \rangle$ . In order to avoid noise amplification during the deconvolution procedure, we have used the fitted data instead of real data. From  $R_i$  we can access the triangular matrix  $\gamma_{ij}^{-1}$ , which is the discrete equivalent of  $\gamma^{-1}$  appearing in (6). Then  $\gamma_{ij}$  is obtained by numerically inverting  $\gamma_{ij}^{-1}$ , and  $\sigma_{ij} = m/\beta(\gamma_{ij} + \gamma_{ji})$ .

It is now a matter of simple data manipulation to obtain the correlation functions  $\langle F_a(0)x(t) \rangle$  and  $\langle \xi(0)x(t) \rangle$ , which are plotted in FIG. 4. As expected, the thermal noise–position correlation function is of the order of  $k_B T$  and decreases to 0 after a time of the order of the second. Besides, the active force–position correlation function has a completely different behavior. First, we observe that the short time behavior exhibits small yet significant negative correlation. This would mean that on average the particle moves *opposite* to the active force for about the first ms. At later times, as expected the correlation function turns positive and grows linearly with time. This confirms the more intuitive idea that the particle on average moves in the same direction of the active forces. However we can now quantify this correlation, which turns out to be of  $10 - 100k_B T$ . The figure also shows that for short time ( $< 1$ s) the effect of thermal noise prevails, while at large times the active force dominates. Again the crossover timescale between these two regimes is of the order of 1 s. This seems to corroborate a scenario for which the short time behavior has equilibrium–like properties, from which the sub-diffusive nature of visco–elasticity emerges, while the long time behavior is strongly governed by active non–equilibrium forces. Finally we briefly comment on the initial anti–correlation exhibited by the active force–position correlation function. This feature appears for the very first milliseconds of the measure-

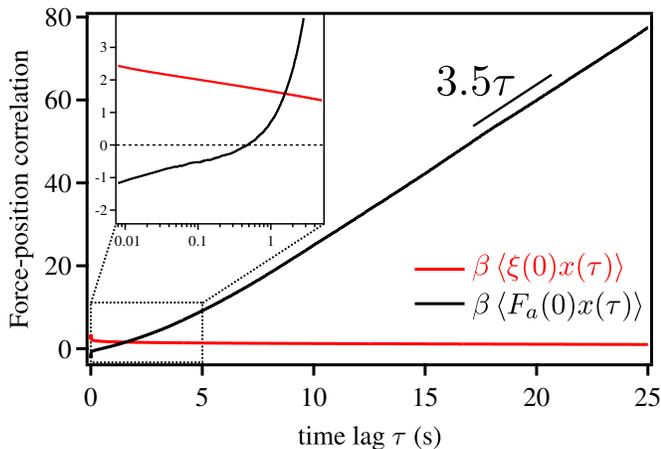


FIG. 4. Position-force correlation as a function of time difference.

ments. We also add that in this time range the data fit (as shown in FIG. 3) is less accurate than at later times. This anti-correlation could however be explained with the help of the depicted scenario. Indeed, if the short time dynamics can be seen as a diffusion in a localized potential determined by the local structure of the cell cortex, the effect of the active forces at these timescales could be a deformation of this potential, which would result in a systematic tilting in the direction opposite to the force. Another useful information that can be extracted from the correlation function concerns the power spent by the active forces on the bead. This can be estimated from the time derivative  $d/dt \langle F_a(0)x(t) \rangle$  when  $t \rightarrow 0$ . Our experimental data shows that this power is about  $30k_B T/s$ . Assuming a ballistic-like behavior, we estimate from FIG. 2 a typical velocity of  $v \sim 5 \times 10^{-3} \mu\text{m/s}$  (compatible with  $0.08 \mu\text{m/s}$  observed in [23]). This leads to an applied force of almost 25 pN, which would mean that a small number (five or less) of motors are contributing to the bead motion.

The equilibrium fluctuation-dissipation theorem relates the response of the system to a small perturbation to spontaneous fluctuations in equilibrium. Out-of-equilibrium extensions involve, besides dissipative aspects, also kinetic aspects where the dynamical activity appears within an extra correlation function with the physical observable under scrutiny. For overdamped Langevin dynamics, and even for dynamics with strong memory effects, the latter is directly related to the non-equilibrium forces driving the system out of equilibrium. Understanding the properties of these forces in the dynamics of living cells is a problem of its own. With this new theoretical tool we have shown how to access some of the properties of these forces. We have been able to quantify the time scale at which active forces driven fluctuations win over thermal ones. Another quantitative spin-off is an estimate for the power dissipated by the active forces into the system, which turns out three orders of magnitude smaller than the chemical power injected into the underlying motors by the ATPase steps. Another conclusion that we have arrived at is the strong

slaving of the probe motion to the active forces, which are coherently followed. We have thus demonstrated that extracting useful information is indeed possible, and this paves the way to a number of improvements or generalizations. An immediate modification of the experimental setup would involve the use of an optical trap to render the unperturbed state stationary, and then pulling on the particle by displacing the trap. Making the system stationary would eliminate time drifts and would simplify the subsequent theoretical analysis by allowing to focus solely on active forces. Other simple improvements of the present work would, for instance, entail tracking higher moments of the bead positions. This could be done by attempting to vary, in the latter stationary setup, the trap's stiffness, instead of the position of its minimum. Even if the rheological meaning of the response function would then be lost, other relevant information concerning fluctuations would then be gained. We leave these projects for future work.

We acknowledge several useful discussions with Julien Tailleur and Damien Robert.

\* Corresponding author: paolo.visco@univ-paris-diderot.fr

- [1] P. Bursac, G. Lenormand, B. Fabry, M. Oliver, D. Weitz, *et al.*, Phys. Rev. Lett. **91**, 198101 (2005).
- [2] C. P. Brangwynne, G. H. Koenderink, F. C. MacKintosh, and D. A. Weitz, Phys. Rev. Lett. **100**, 118104 (2008).
- [3] J. Prost, J.-F. Joanny, and J. M. R. Parrondo, Phys. Rev. Lett. **103**, 090601 (2009).
- [4] F. Gallet, D. Arcizet, P. Bohec, and A. Richert, Soft Matter **5**, 2947 (2009).
- [5] D. Mizuno, C. Tardin, C. Schmidt, and F. MacKintosh, Science **315**, 370 (2007).
- [6] M. Falcioni, S. Isola, and A. Vulpiani, Phys. Lett. A **144** (1990); M. Falcioni and A. Vulpiani, Physica A **215**, 481 (1995).
- [7] T. Speck and U. Seifert, Europhys. Lett. **74**, 391 (2006).
- [8] U. M. B. Marconi, A. Puglisi, L. Rondoni, and A. Vulpiani, Physics Reports **461**, 111 (2008).
- [9] D. Villamaina, A. Puglisi, and A. Vulpiani, J. Stat. Mech. **L10001** (2008); D. Villamaina, A. Baldassarri, A. Puglisi, and A. Vulpiani, *ibid.* **P07024** (2009).
- [10] R. Chetrite, G. Falkovich, and K. Gawedzki, J. Stat. Mech. **P08005**, (2008).
- [11] T. Speck and U. Seifert, Phys. Rev. E **79**, 040102(R) (2009).
- [12] M. Baiesi, C. Maes, and B. Wynants, Phys. Rev. Lett. **103**, 010602 (2009); J. Stat. Phys. **137**, 1094 (2009); Proc. R. Soc. A **467**, 2792 (2011); M. Baiesi, E. Boksbojm, C. Maes, and B. Wynants, J. Stat. Phys. **139**, 492 (2010).
- [13] U. Seifert and T. Speck, Europhys. Lett. **89**, 10007 (2010).
- [14] V. Blickle, T. Speck, L. Helden, U. Seifert, and C. Bechinger, Phys. Rev. Lett. **96**, 070603 (2006).
- [15] V. Blickle, T. Speck, C. Lutz, U. Seifert, and C. Bechinger, Phys. Rev. Lett. **98**, 210601 (2007).
- [16] J. Gomez-Solano, A. Petrosyan, S. Ciliberto, R. Chetrite, and K. Gawedzki, Phys. Rev. Lett. **103**, 040601 (2009); J. R. Gomez-Solano, A. Petrosyan, S. Ciliberto, and C. Maes, J. Stat. Mech. **2011**, P01008 (2011).
- [17] T. Harada and S. Sasa, Phys. Rev. E **73**, 026131 (2006).
- [18] L. Cugliandolo, J. Kurchan, and G. Parisi, J. Phys. I France **4**,

- 1641 (1994).
- [19] P. Bohec *et al.*, In preparation.
- [20] B. Fabry, G. N. Maksym, J. P. Butler, M. Glogauer, D. Navajas, and J. J. Fredberg, *Phys. Rev. Lett.* **87**, 148102 (2001).
- [21] M. Balland, N. Desprat, D. Icard, S. Féréol, A. Asnacios, *et al.*, *Phys. Rev. E* **74**, 021911 (2006).
- [22] D. Robert, T.-H. Nguyen, F. Gallet, and C. Wilhelm, *PLoS ONE* **5**, e10046 (2010).
- [23] J. T. Finer, R. M. Simmons, and J. A. Spudich, *Nature* **368**, 113 (1994).