

Fluctuation-response relation for a nonequilibrium system with resolved Markovian embedding

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Fluctuation-response relations must be modified to describe nonequilibrium systems with non-Markovian dynamics. Here, we experimentally demonstrate that such relation is quantitatively recovered when the appropriate Markovian embedding of the dynamics is explicitly resolved. Using a colloidal particle optically trapped in a harmonic potential and driven out of equilibrium by a controlled colored noise, we study the response to a perturbation of the stiffness of the confining potential. While the reduced dynamics violates equilibrium fluctuation-response relations, we show that the dynamical response to the stiffness perturbation is fully determined by steady-state correlations involving the exact conjugate observable in the Markovian embedding.

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I. INTRODUCTION

For a system in thermal equilibrium, fluctuation-response relations (FRRs) provide a direct connection between the spontaneous fluctuations of an observable and its response to an external perturbation [1–4]. In the canonical example, the autocorrelation function of the position $x(t)$ of a diffusing object determines the dissipative mean response $\langle x(t) \rangle$ following the switching off of a constant force [5]. Many natural and artificial systems, however, experience perturbations of a different nature, extending beyond the constant-force paradigm. Cells, for instance, are frequently exposed to dynamic mechanical changes in their environment [6] or in their internal mechanical properties [7]. A sudden stiffening of the substrate constitutes a perturbation not in the form of an applied external force, but rather through a modification of the mechanical properties of the environment, which triggers a dynamical response, such as increased cell spreading. More broadly, cellular adaptation through the recovery of homeostasis exemplifies complex perturbations that modify the system's properties and connect states characterized by different free energies and timescales [8]. Such responses, which combine nonequilibrium dynamics and complex perturbations, can be challenging to predict.

This motivates the search for explicit forms of FRRs applicable to broader classes of perturbations. A recent step in this direction is the experimental verification of an FRR adapted

to a perturbation of the stiffness of a harmonic potential [10]. While this FRR correctly predicts the response to a dynamical compression, it breaks down when the system is driven into nonequilibrium states by colored noise, highlighting the limits of equilibrium-inspired FRRs. A second step toward predicting the response of complex biophysical processes is therefore to derive FRRs for systems driven out of equilibrium and subject to active fluctuations. In biophysics, these effective stochastic forces originate from active processes [11,12] which can be reproduced in model experiments using optical traps [9,13,14]. The active force acts as an additional “hidden” degree of freedom which induces non-Markovian dynamics [15]. In that case, since the friction force of the surrounding fluid generally remains instantaneous, correlated forces drive the system out of equilibrium [12]. Importantly, non-Markovianity induced by hidden degrees of freedom also occurs in equilibrium systems, for example, in complex fluids [16,17]. In that case, noise correlation and friction kernel match, highlighting that memory effects alone do not imply nonequilibrium. The influence of unresolved dynamics on observable time series is closely related to the problem of partial observations formalized in state estimation theory [18,19]. Since the timescales at play in driven nonequilibrium systems are usually not well separated, one cannot rely on an effective temperature with clearly defined regimes [20]. FRRs for nonequilibrium states have been derived using generalized conjugated variables [21–23]. Experimental demonstrations have been performed in the case of deterministic nonequilibrium driving, where a comoving equilibriumlike frame of reference can be defined [24,25], and in a biophysical context with partially observable dynamics [26]. Even when hidden degrees of freedom cannot be measured, an inequality bounding the response can be recovered [27]. When possible, recovering FRRs for nonequilibrium systems allows one to predict the evolution of a system following a perturbation, based on the analysis of its spontaneous fluctuations in steady

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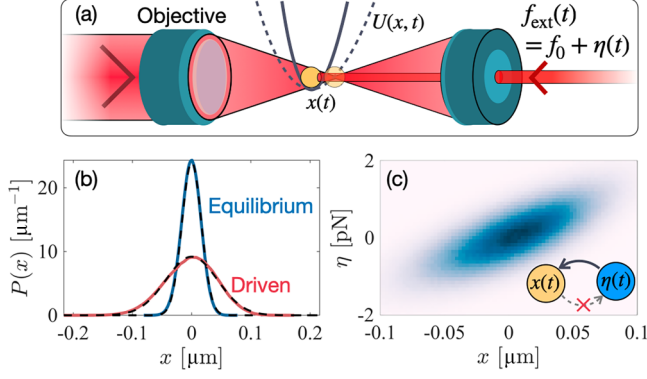


FIG. 1. (a) Schematic view of the experimental setup: a micron-sized particle suspended in water is optically trapped by a focused laser beam. It is subjected to a time-dependent external forcing $f_{\text{ext}}(t)$, imposed by the radiation pressure of a secondary laser beam. The position $x(t)$ along the optical axis is recorded via the diffraction of a third low-power laser beam (not represented). Further details are provided in Appendix A and in Refs. [9,10]. (b) Experimentally measured positional probability density functions $P(x)$ in thermal equilibrium (blue) and in the presence of colored noise driving (red), together with the respective analytical Gaussian distributions (black dashed line). (c) Experimentally measured bivariate distribution $p(x, \eta)$ maintained out of equilibrium by the nonreciprocal coupling between both variables, as sketched in the inset.

state. Generalized FRRs, including complex perturbations, therefore lead to improved control of systems across scales, ranging from biophysics [26] to granular materials [28] and climate dynamics [29], where predicting the response to perturbations becomes critical.

Here, we employ a generalization of FRR that holds for a system driven out of equilibrium by a correlated stochastic force [21] and focus on perturbation in the form of a change in stiffness, i.e., the mechanical properties of the confining potential, which defines the system's timescale and free energy [10]. The relation between fluctuations and response relies on the identification of the appropriate conjugate variable, which takes here a nontrivial form. We show this relation experimentally by trapping a colloidal particle in a time-dependent optical potential under the action of correlated, noisy external forces. In contrast with often unmeasurable biological active driving forces [26], our setup allows us to measure simultaneously the position of the particle and the external noise, probing experimentally the Markovian embedding. This model experiment reproduces the dynamics of minimalist active matter systems, while offering the possibility of precise control of the potential stiffness. Our results show that, upon monitoring the correct conjugate variable, it is possible to predict the response of an active system to a mechanical transformation. We also provide a formal derivation of the generalized FRR, solely based on the hypothesis of Markovianity of the system.

II. RESPONSE TO A PERTURBATION OF THE STIFFNESS

In our experimental setup [see Fig. 1(a)], a single colloidal particle is trapped in a harmonic optical trap with stiffness $\kappa(t)$ which is modulated over time by controlling the intensity

of the trapping laser [10,30,31]. Such optical stiffness change forms a fundamental thermodynamic building block for realizing microscopic analogues of heat engines [32,33]. A secondary laser beam imposes an external radiation pressure force $f(t) = f_0 + \eta(t)$, sum of a constant contribution f_0 , and a zero-mean exponentially correlated noise $\eta(t)$, with correlation function $\langle \eta(t)\eta(s) \rangle = \sigma_\eta^2 e^{-|t-s|/\tau_c}$ with correlation time τ_c . The contribution of the constant force f_0 vanishes by subtracting the mean of the recorded trajectories. The overall dynamics of the system can therefore be described as a nonreciprocally (unidirectionally) coupled pair of equations, for the variables x and η that reads

$$\begin{aligned}\dot{x}(t) &= -\frac{\kappa(t)}{\gamma}x(t) + \sqrt{2D}\xi(t) + \frac{1}{\gamma}\eta(t), \\ \dot{\eta}(t) &= -\frac{1}{\tau_c}\eta(t) + \sqrt{2D_\eta}\theta(t),\end{aligned}\quad (1)$$

where γ is Stokes drag coefficient, $D = k_B T / \gamma$ is the thermal diffusion coefficient with k_B being the Boltzmann constant, and $D_\eta = \sigma_\eta^2 / \tau_c$ is the diffusion coefficient of the noise's dynamic. $\xi(t)$ and $\theta(t)$ are uncorrelated zero-mean white noises. The stiffness of the potential and the viscous drag define the system's characteristic relaxation time $\tau_r = \gamma / \kappa$. The nonreciprocal coupling between the variables x and η drives the system in a nonequilibrium steady state (NESS) [34], modifying its response properties [15,35,36]. Yet the Gaussian nature of the driving noise $\eta(t)$ maintains the Gaussian statistics $P(x)$ for $x(t)$ in the driven system, simply characterized by a larger variance than in the absence of driving [Fig. 1(b)] which can be captured as an effective temperature [10].

The reduced dynamics $x(t)$ is non-Markovian due to the memory contained in the colored noise. It also vividly reveals its nonequilibrium nature by breaking the FDR of the second kind [2]: the viscous friction force is memoryless while the noise is correlated, as observed for bacterial baths [12]. Yet the possibility to record the time series of position and noise simultaneously allows us to study the two-dimensional embedding $\{x(t), \eta(t)\}$, which is a Markovian nonequilibrium system [15]. The bivariate distribution $p(x, \eta)$ corresponds to a Gaussian NESS with nonvanishing correlations between both variables [Fig. 1(c)] and stationary currents.

By controlling the intensity of the trapping beam, we impose an abrupt change in stiffness, from $\kappa_i = 13.4 \pm 0.11$ to $\kappa_f = 14.6 \pm 0.13$ pN/ μm [Fig. 2(a)] of small amplitude. This cycle consisting of compression followed by its time-reversed expansion from κ_f to κ_i is applied sequentially to the trapped microparticle, with a waiting time between each repetition larger than all timescales $\tau_i = \gamma / \kappa_i$, $\tau_f = \gamma / \kappa_f$, and τ_c . We record the time series $x(t)$ of positions of the particle subjected to this series of protocols and split it into subtrajectories centered around each protocol, relying on the ergodicity of the subtrajectories [37]. This leads to an ensemble of *circa* 18 000 independent trajectories experiencing the same compression. Our experimental setup, therefore, enables the collection of large statistics similar to numerical simulations, with high temporal resolution. Both are generally challenging in biological experiments. The response of the ensemble $\{x_j(t)\}$ to the compression $\kappa(t)$ is determined by its positional probability density $P(x, t)$. Importantly, since at all times $P(x, t)$ is a

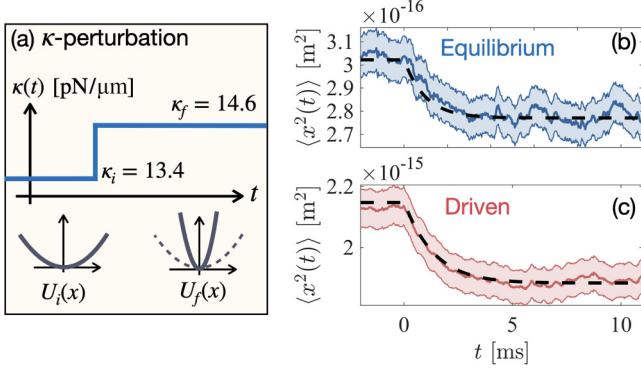


FIG. 2. (a) Schematic representation of the perturbation applied: a sudden change of the stiffness κ of the optical potential $U(x) = \kappa x^2/2$ trapping the particle. (b) Time-dependent second moment $\langle x^2(t) \rangle$ following a sudden increase in κ in the absence of driving noise, together with the analytical simple exponential decay (black dashed line). (c) Time-dependent second moment in the presence of exponentially correlated noise with constant variance and correlation time, together with the analytical biexponential decay (black dashed line).

centered Gaussian distribution, it is fully characterized by its second moment $\sigma^2(t) = \langle x^2(t) \rangle$.

In Fig. 2(b), we show the evolution of $\sigma^2(t)$ for a stiffness perturbation in the absence of driving noise $\eta(t)$. At the beginning of the cycle, the system is at thermal equilibrium and its second moment obeys the equipartition theorem in the harmonic potential: $\sigma_i^2 \equiv \langle x^2(t < 0) \rangle = k_B T / \kappa_i$. At the end of the cycle, the system reaches equilibrium again, with $\sigma_f^2 \equiv \langle x^2(t \gg \tau_f) \rangle = k_B T / \kappa_f$. Its dynamical evolution for finite times agrees well with the simple exponential decay predicted by solving the Fokker-Planck equation in a harmonic potential $\sigma^2(t) = \sigma_i^2 + (\sigma_f^2 - \sigma_i^2)e^{-2t/\tau_f}$ [38]. In Fig. 2(c), we show the response to the same stiffness perturbation with a colored noise driving $\eta(t)$ with $\tau_c = 3$ ms. In that case as well, the system starts and ends in a Gaussian steady state, but the respective second moment does not obey the equipartition theorem [9,39]. The evolution of $\langle x^2(t) \rangle$ under the influence of both timescales, τ_f and τ_c , differs from a simple exponential decay. It agrees well with the analytical solution for a colored-noise driven Ornstein-Uhlenbeck process (given in Appendix B), with mixed timescales τ_i , τ_f , and τ_c . In the following, we ask if these time evolutions after a κ perturbation can be predicted from steady-state measurements.

III. EXPERIMENTAL VERIFICATION OF THE GENERALIZED FRR

At thermal equilibrium, the system's response to an external perturbation is directly related to its equilibrium correlation function via the temperature T . Here, a stiffness perturbation leads to a response of the observable x^2 and the appropriate FRR should connect $\mathcal{R}(t) \equiv \sigma^2(t) - \sigma_f^2$ to the correlation function $\langle x^2(t)x^2(0) \rangle_f$, where $\langle \dots \rangle_f$ denotes an average in the final steady state. Using Wick's theorem, the latter can be expressed as a function of the square of the positional correlation function $C_{xx}(t) = \langle x(0)x(t) \rangle_f$, leading

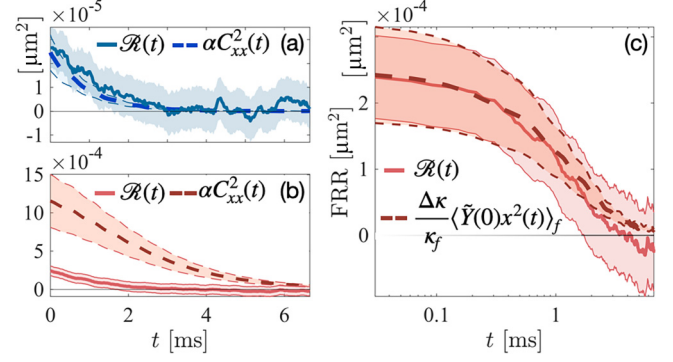


FIG. 3. (a) FRR relation Eq. (2) for a system in thermal equilibrium, probed via a stiffness perturbation. The response function $\mathcal{R}(t)$ (blue line) agrees with the scaled squared correlation function (dark blue dashed line). (b) The same FRR breaks in the presence of colored-noise driving as shown in Ref. [10]. (c) Generalized FRR Eq. (4) taking into account both position and noise variable: the response function $\mathcal{R}(t)$ (red line) agrees with the cross correlation between the observable x^2 and its exact conjugate variable Y , multiplied by κ_f (dark red dashed line).

to the following FRR for a stiffness perturbation:

$$\mathcal{R}(t) = \frac{\Delta\kappa}{k_B T} \left(1 + \frac{\Delta\kappa}{\kappa_i} \right) C_{xx}^2(t) \quad (2)$$

with a first and second order contribution in the perturbation amplitude $\Delta\kappa = \kappa_f - \kappa_i$ [10]. In Fig. 3(a), we show that this FRR is fulfilled for a stiffness change between two equilibrium states: the response and correlation function share the same time-dependence and their relative amplitude is dictated by the prefactor $\alpha = \frac{\Delta\kappa}{k_B T} (1 + \frac{\Delta\kappa}{\kappa_i})$. In Fig. 3(b), we, however, show that, in the presence of colored noise, the FRR is broken. The response and correlation have distinct amplitudes, but most importantly, different time dependencies, as was already demonstrated in Ref. [10] for this case. As a consequence of this, no constant effective temperature can allow to recover the FRR but instead, a time-dependent effective temperature is necessary to enforce the equality of $\mathcal{R}(t)$ and $\alpha C_{xx}^2(t)$ at all times [40] and the thermodynamic meaning of such temperature in a system with mixed timescales is not clear [28,41]. This behavior reflects the non-Markovian nature of the reduced dynamics $x(t)$ [42].

In contrast, the bidimensional embedding Eq. (1) provides a Markovian description which allows generalized FRR to be applied. This is conditioned on the identification of the correct conjugate variable $Y(x, \eta)$ to the perturbation applied [21,26,43]. The response function of a given observable is then related to the correlation of Y with the same observable [21]. The conjugate variable is defined from the Markovian-embedding steady-state $p(x, \eta)$ and, for a small perturbation, can be linearized

$$Y(x, \eta) = \frac{1}{p(x, \eta)} \frac{\partial p(x, \eta)}{\partial \kappa}. \quad (3)$$

We further define the dimensionless conjugate variable $\tilde{Y} = \kappa_f Y$, leading to the following generalized FRR

$$\mathcal{R}(t) = \frac{\Delta\kappa}{\kappa_f} \langle \tilde{Y}(0)x^2(t) \rangle_f \quad (4)$$

derived in Appendix C relying only on the assumption of Markovian dynamics and on the existence of a steady state. The result Eq. (4) is therefore valid for a very large class of systems, beyond our experimental verification. This result is equivalent to Eq. (5) in Ref. [21] using x^2 as an observable, instead of the conjugate variable itself. As shown in Ref. [23], the generic form of the conjugated variable Y is related to entropy [44]. Here, the entropy is defined by the bivariate PDF $s_{\text{sys}} = -k_B \ln(p[x, \eta])$. In the general case, the conjugate variable can be evaluated directly from the measured histograms [43]. Here, the bivariate state $p(x, \eta)$ can be expressed as a function of x and η [45], which leads to an explicit expression of $\tilde{Y}$. This allows to express the r.h.s. of Eq. (4) as a function of three time-dependent correlation functions $\langle x^2(0)x^2(t) \rangle_f$, $\langle x(0)\eta(0)x^2(t) \rangle_f$ and $\langle \eta^2(0)x^2(t) \rangle_f$, each evaluated in the final NESS. The result is a simple yet lengthy equation, which is presented in Appendix D. Each correlation function can be measured experimentally and is shown in Fig. 5 (Appendix D), in good agreement with the result of numerical simulations. This explicit form of r.h.s. of Eq. (4) allows to test experimentally the generalized FRR with high resolution.

In Fig. 3(c) we show the response function $\mathcal{R}(t)$ together with the scaled correlation function $\frac{\Delta\kappa}{\kappa_f} \langle \tilde{Y}(0)x^2(t) \rangle_f$ of the conjugate variable $\tilde{Y}$ with the observable $x^2(t)$. The agreement between both quantities shows that the generalized FRR [Eq. (4)] holds when the appropriate conjugate variable is evaluated in the Markovian embedding. This bivariate description is sufficient to recover a simple relation between the response of the observable x^2 of the system to a mechanical change in κ and its correlation with the conjugate variable $\tilde{Y}$. The practical implication of this result is the ability to predict the complex time evolution of the system after a stiffness perturbation in the presence of colored noise. This prediction relies on the evaluation of the appropriate correlation function, implying a nontrivial conjugate variable.

IV. CONCLUSION

In this work, we experimentally demonstrate how generalized fluctuation-response relation applies to nonequilibrium systems when the appropriate Markovian embedding is resolved. Our experimental setup, which involves an optically trapped microparticle driven by colored noise, enables us to fully resolve the required Markovian embedding. We focus on the system's response to a mechanical change in stiffness, which modifies the system's state beyond the mean position. This type of perturbation applied to a nonequilibrium state represents a first step toward describing dynamical changes in microbiological processes. It demonstrates that resolving the Markovian embedding enables to predict the nonequilibrium response from steady-state fluctuations, even for a complex system subjected to a change in mechanical stiffness. More generally, this validation supports the use of Markovian embeddings as an operational basis to apply FRRs to nonequilibrium systems in which the additional degrees of freedom are not directly accessible but can be inferred from measured trajectories [26].

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DATA AVAILABILITY

All experimental data used in the work as well as the codes necessary to perform the present analysis are available in an open repository [46].

APPENDIX A: EXPERIMENTAL SETUP

Our experimental setup consists of a 3 μm polystyrene microsphere optically trapped in deionized water at room temperature [9]. The harmonic potential is induced by focusing inside the cell a linearly polarized Gaussian beam (785 nm, CW 110 mW laser diode, Coherent OBIS) through a high numerical aperture (NA) objective (Nikon Plan Apo VC, 60 $\times$, NA = 1.20 water immersion). An additional force in the form of radiation pressure is applied to the sphere using a time-dependent fraction of the light-beam emitted by an additional high-power laser (800 nm, CW 5 W Ti:Sa laser, Spectra Physics 3900S)—see Figs. 1 and 4. An additional time-dependent external force is applied via radiation pressure from a second high-power laser beam (800 nm, CW 5 W Ti:Sa laser, Spectra Physics 3900S), weakly focused in order to avoid inducing gradient forces (sent on the microsphere as a thin beam, strongly underfilling the aperture of a lower-NA objective—Nikon Plan Fluor Extra Large Working Distance, 60 $\times$, NA = 0.7) [9,10,31]. The intensity of both the trapping laser and the radiation pressure beam are controlled by two independent acousto-optic modulators (Gooch and Housego 3200) using a digital-to-analogue card (NI PXIe 6361) and a PYTHON code. This configuration enables independent tuning of the trap stiffness and the amplitude of the applied external force.

The instantaneous position $x(t)$ of the sphere along the optical axis is measured by recording the light scattered off the sphere of a low-power 639 nm laser (CW 30 mW laser diode, Thorlabs HL6323MG), sent on the bead via the second, low-NA objective. The scattered light is collected by Obj1 and recorded by a photodiode (100 MHz, Thorlabs Det10A). The signal is amplified (SR560, Stanford Research), filtered (0.3 Hz high-pass filter at 6 dB/oct to remove the DC component and through a 100 kHz low-pass filter at 6 dB/oct), and digitized (NI PCI-6251). For small displacements, the detected voltage is linearly related to the bead position, ensuring operation within the linear response regime, allowing a direct calibration. The AOM controlling the trapping laser

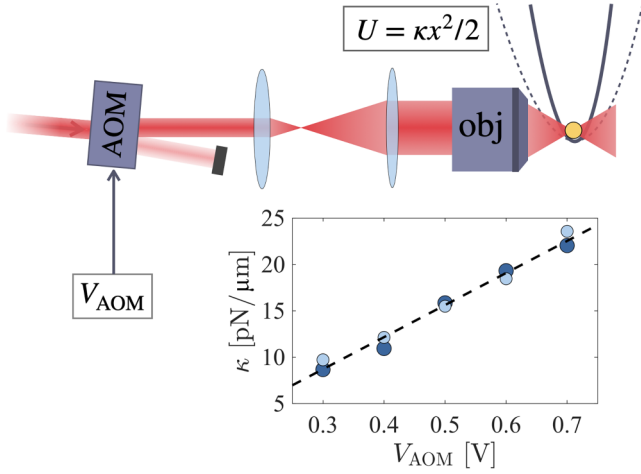


FIG. 4. Schematic view of the calibration of the optical potential. The trapping laser is sent through an acousto-optic modulator (AOM). The AOM is fed with a voltage V_{AOM} which controls the intensity of the first order diffracted beam, which is sent into a high NA objective to form the optical potential. Modifying V_{AOM} leads to a change in the trap stiffness κ . The linear relation between κ and V_{AOM} is calibrated (lower right inset), allowing to control precisely the stiffness of the potential.

is calibrated directly from the corner frequency of the power spectral density of the recorded bead dynamics (see Fig. 4), allowing for a control of the stiffness κ .

The experimental results are complemented by numerical simulations of the Langevin dynamics. Both variables $x(t)$ and $\eta(t)$ obey first order stochastic differential equations, which can be numerically simulated using standard Euler-Maruyama approximation.

APPENDIX B: RESPONSE FUNCTION IN THE PRESENCE OF COLORED NOISE

As shown in Ref. [10], the time evolution of the second moment $\sigma^2(t) = \langle x^2(t) \rangle$ after a change of stiffness from κ_i to κ_f reads

$$\begin{aligned} \sigma^2(t) = & \left(D\tau_i + \frac{\sigma_\eta^2 \tau_i^2 \tau_c}{\gamma^2(\tau_i + \tau_c)} \right) e^{-2t/\tau_f} + D\tau_f(1 - e^{-2t/\tau_f}) \\ & + \frac{\sigma_\eta^2}{\gamma^2} \left(\frac{\tau_f^2 \tau_c}{\tau_f + \tau_c} + \frac{2\tau_f^2 \tau_c}{\tau_f - \tau_c} e^{-2t/\tau_f} - \frac{2\tau_f^2 \tau_c^2}{\tau_f^2 - \tau_c^2} e^{-t(\frac{1}{\tau_f} + \frac{1}{\tau_c})} \right) \\ & + \frac{2\sigma_\eta^2 \tau_i \tau_f \tau_c}{\gamma^2(\tau_i + \tau_c)(\tau_f - \tau_c)} \left(e^{-t(\frac{1}{\tau_f} + \frac{1}{\tau_c})} - e^{-2t/\tau_f} \right), \end{aligned} \quad (\text{B1})$$

where $\tau_f \equiv \gamma/\kappa_f$. This enters the response function $\mathcal{R}(t) = \sigma^2(t) - \sigma_f^2$. In the absence of colored noise σ_η , one retrieves the standard result $\sigma^2(t) = (\sigma_i - \sigma_f)e^{-2t/\tau_f} + \sigma_f$ valid for a transient between two equilibrium states.

APPENDIX C: GENERALIZED FLUCTUATION-RESPONSE RELATION FOR MARKOVIAN DYNAMICS

In this Appendix, we consider a simple generalization of a fluctuation-dissipation relation within a class A approach

according to the classification of Ref. [4]. The derivation follows the one discussed in the Supplemental Material of Ref. [22], using notations compatible with the rest of the present work. The FRR which we derive in this way is related to the FDT of Prost *et al.* [21] obtained via a different approach. The only necessary assumptions for our derivation are the Markovian nature of the system's evolution, and the existence of the stationary state for any magnitude of perturbation in the admissible range.

Parallel to the standard discussion for the equilibrium, we prepare a system in a stationary state under the action of the constant perturbation ϕ , which is switched off at time $t = 0$, after which the system undergoes autonomous evolution. The stationary state under constant perturbation ϕ is characterized by the probability density function (PDF) $p(\mathbf{x}|\phi)$, where $\mathbf{x}$ is a vector of internal state variables of the system. The number of these variables must be taken to be sufficient for a Markovian description of its time evolution. In our case, the perturbation is a stiffness modification $\phi = (\kappa_f - \kappa_i)$.

The PDF of $\mathbf{x}$ at time t after switching of the perturbation ϕ is given by

$$p(\mathbf{x}; t) = \int p(\mathbf{x}, t|\mathbf{x}'; 0) p(\mathbf{x}'|\phi) d\mathbf{x}',$$

where $p(\mathbf{x}, t|\mathbf{x}'; t')$ denotes the transition probability density from the state $\mathbf{x}'$ at time $t' < t$ to the state $\mathbf{x}$ at time t in the absence of perturbation. Now we consider a single-time observable $\mathcal{O}(t) \equiv \mathcal{O}[x(t)]$. Its mean value at time t is given by

$$\langle \mathcal{O}(t) | \phi \rangle = \iint \mathcal{O}(\mathbf{x}) p(\mathbf{x}, t|\mathbf{x}', 0) p(\mathbf{x}'|\phi) d\mathbf{x} d\mathbf{x}'.$$

This mean is then compared to a reference state, in which the system is prepared without perturbation (i.e., with $\phi = 0$):

$$\langle \mathcal{O}(t) | 0 \rangle = \iint \mathcal{O}(\mathbf{x}) p(\mathbf{x}, t|\mathbf{x}', 0) p(\mathbf{x}'|0) d\mathbf{x} d\mathbf{x}'.$$

The response of the system to the perturbation is thus given by

$$\begin{aligned} \langle \mathcal{O}(t) | \phi \rangle - \langle \mathcal{O}(t) | 0 \rangle = & \iint d\mathbf{x} d\mathbf{x}' \mathcal{O}(\mathbf{x}) p(\mathbf{x}, t|\mathbf{x}', 0) \\ & \times [p(\mathbf{x}'|\phi) - p(\mathbf{x}'|0)]. \end{aligned}$$

Introducing

$$\mathcal{Y}(\mathbf{x}, \phi) = \frac{p(\mathbf{x}|\phi) - p(\mathbf{x}|0)}{p(\mathbf{x}|0)}$$

we thus may write

$$\begin{aligned} \langle \mathcal{O}(t) | \phi \rangle - \langle \mathcal{O}(t) | 0 \rangle = & \iint d\mathbf{x} d\mathbf{x}' \mathcal{O}(\mathbf{x}) \mathcal{Y}(\mathbf{x}', \phi) p(\mathbf{x}, t|\mathbf{x}', 0) p(\mathbf{x}'|0) \\ = & \iint d\mathbf{x} d\mathbf{x}' \mathcal{O}(\mathbf{x}) \mathcal{Y}(\mathbf{x}', \phi) p(\mathbf{x}, t; \mathbf{x}', 0), \end{aligned}$$

where $p(\mathbf{x}, t; \mathbf{x}', 0)$ is the joint PDF of internal variables at times t and 0. Thus,

$$\langle \mathcal{O}(t) | \phi \rangle - \langle \mathcal{O}(t) | 0 \rangle = \langle \mathcal{O}(t) \mathcal{Y}(\phi, 0) \rangle_0. \quad (\text{C1})$$

By writing $\mathcal{Y}(\phi, 0)$ we stress that the variable $\mathcal{Y}$ (the exact conjugate variable to the perturbation) is calculated or measured at $t = 0$. The subscript 0 in the last mean indicates that this one is calculated using the joint PDF in the absence of the perturbation. Note that $\mathcal{Y}$ itself is still dependent on the perturbation strength. Equation (C1) is the nonlinear fluctuation-dissipation theorem of Ref. [43].

Now, let us assume that $p(\mathbf{x}|\phi)$ is differentiable w.r.t. ϕ , and approximate $\mathcal{Y}(\mathbf{x}, \phi)$ in the linear order:

$$\begin{aligned}\mathcal{Y}(\mathbf{x}, \phi) &\simeq \frac{1}{p(\mathbf{x}|0)} \frac{d}{d\phi} p(\mathbf{x}|\phi)|_{\phi=0} \phi \\ &= \phi \nabla_{\phi} \ln p(\mathbf{x}, \phi)|_{\phi=0} \equiv \phi Y(\mathbf{x}),\end{aligned}$$

where the variable $Y(\mathbf{x})$ is now independent from the perturbation's magnitude. We then get

$$\langle \mathcal{O}(t)|\phi \rangle - \langle \mathcal{O}(t)|0 \rangle = \phi \langle \mathcal{O}(t)Y(0) \rangle_0. \quad (\text{C2})$$

Equation (C2) is the customary FRR in a stationary state (equilibrium or not). Taking $\mathcal{O}(\mathbf{x}) = Y(\mathbf{x})$ we get the FDT of Prost *et al.* [21] (up to a different sign convention). This FRR is pertinent to the linear response case.

In equilibrium, this relation

$$\langle Y(t)|\phi \rangle - \langle Y(t)|0 \rangle = \phi \langle Y(t)Y(0) \rangle_0$$

is the standard FDT. In our case, focusing on the observable $\mathcal{O}(t) = x^2$ leads to

$$\langle x^2(t)|\phi \rangle - \langle x^2(t)|0 \rangle = \phi \langle x^2(t)Y(0) \rangle \quad (\text{C3})$$

with the l.h.s. being the response function of x^2 to the perturbation ϕ .

APPENDIX D: GENERALIZED FRR FOR A TRAPPED BROWNIAN PARTICLE UNDER COLORED NOISE

The conjugate variable $\tilde{Y}(x, \eta)$ given by Eq. (3) depends on the bivariate state $p(x, \eta)$ which is known in our case [26,45]. This allows for the direct expression

$$\tilde{Y}(x, \eta) = a_0 + a_{11}x^2 + a_{12}x\eta + a_{22}\eta^2 \quad (\text{D1})$$

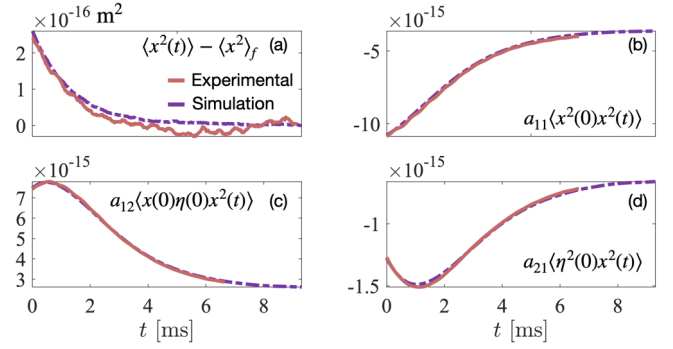


FIG. 5. Experimental results (red solid lines) and results of numerical simulation with the experimental parameters (purple dashed-dotted lines) in m^2 for (a) the response function $\mathcal{R}(t)$, (b) the scaled autocorrelation $\langle x^2(0)x^2(t) \rangle_f$, (c) the scaled cross correlation $\langle x(0)\eta(0)x^2(t) \rangle_f$, and (d) the scaled cross correlation $\langle \eta^2(0)x^2(t) \rangle_f$, the latter three being evaluated in the final steady-state.

with coefficients [using $M \equiv D\gamma^2(\tau_f + \tau_c)^2 + \sigma_\eta^2\tau_f^2\tau_c$],

$$\begin{aligned}a_0 &= \frac{D\gamma^2(\tau_f + \tau_c)^3 + \sigma_\eta^2\tau_f\tau_c^2(\tau_f + 3\tau_c)}{2(\tau_f + \tau_c)M}, \\ a_{11} &= -\frac{\gamma^2(\tau_f + \tau_c)(D\gamma^2[\tau_f + \tau_c]^3 + \sigma_\eta^2\tau_f\tau_c^2[\tau_f + 3\tau_c])}{2\tau_f M^2}, \\ a_{12} &= \frac{\gamma\tau_f\tau_c(D\gamma^2[\tau_f + \tau_c]^2 + \sigma_\eta^2\tau_f\tau_c[\tau_f + 2\tau_c])}{M^2}, \\ a_{22} &= -\frac{D\gamma^2\tau_f\tau_c^2(\tau_f^2 - \tau_c^2) + \sigma_\eta^2\tau_f^3\tau_c^3}{2M^2}.\end{aligned}$$

Using this form of the conjugate variable in cross correlation $\langle \tilde{Y}(0)x^2(t) \rangle$ leads to the generalized FRR

$$\begin{aligned}\mathcal{R}(t) &= \frac{\Delta\kappa}{\kappa_f} [a_0 \langle x^2(t) \rangle_f + a_{11} \langle x^2(0)x^2(t) \rangle_f \\ &\quad + a_{12} \langle x(0)\eta(0)x^2(t) \rangle_f + a_{22} \langle \eta^2(0)x^2(t) \rangle_f]. \quad (\text{D2})\end{aligned}$$

The response function $\mathcal{R}(t)$ as well as the three cross correlations on the r.h.s. of Eq. (D2) can be measured experimentally (Fig. 5). The correlation functions are evaluated as mixed time and ensemble average over the ensemble of trajectories in the final steady state. The experimental results are complemented by numerical simulations of the bivariate process, Eq. (1), using the experimentally measured parameters. The explicit form of the FRR [Eq. (D2)] composed of these measured cross correlations is the result shown in Fig. 3(c) in the main text.

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