Journal Club for Condensed Matter Physics
https://www.condmatjclub.org
DOI:10.36471/JCCM_July_2023_02

Two steps forward – and one step back?

Measuring fluctuation-dissipation breakdown from fluctuations only

Onsager regression characterizes living systems in passive measurements
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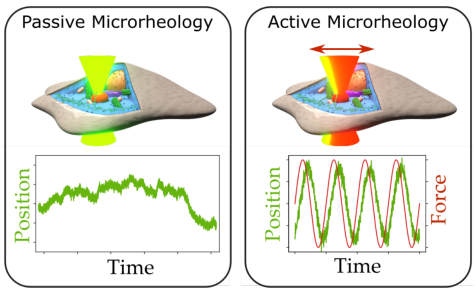
bioRxiv 2022.05.15.491928

Recommended with a Commentary by Pierre Ronceray, Aix-Marseille Université, CNRS, CINaM, Turing Center for Living Systems, Marseille, France

In the past decade, the characterization of the nonequilibrium properties of intracellular objects and tracers have focused a lot of attention from the statistical physics community. A flurry of methods were developed to characterize the breakdown of detailed balance [1, 2] that result from the active, energy-consuming cellular processes, as well as the associated production of entropy through measurements of the nonequilibrium currents [3, 4] and their fluctuations [5, 6]. This quest for efficient methods to quantify how much a system is out-of-equilibrium from passive observations of their trajectories has sometimes resulted in beautiful new statistical physics [5]. However, in my opinion, the applications of these methods to biological systems have been underwhelming: they have yet to yield any new insights on cell biology.

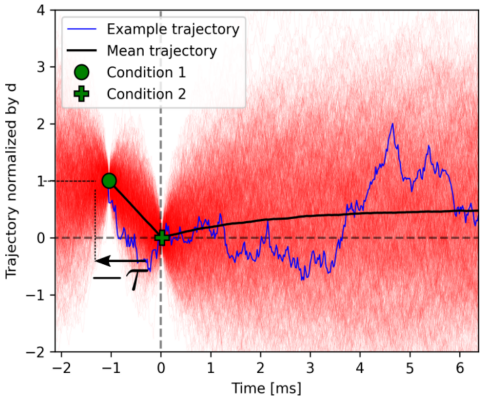


Indeed, a key limitation is that these techniques rely on the quantification of probability currents within observable quantities. However, in general the dissipation corresponding to *observable* currents only represents a faint trace of the total activity of the system – while the lion's share occurs within non-observed quantities, which cannot be tackled with existing techniques (although some effort was made for discrete systems [7]). To capture nonequilibrium dynamics, one thus has to get back to the traditional approach consisting in studying breakdown of the fluctuation-dissipation relation (FDR) by combining passive micrheology experiments to characterize spontaneous fluctuations, with active microrheology measurements whereby the response of the tracer to external forces is probed using optical tweezers. While the passive measurements are easily performed, the active ones are particularly tricky, which precludes wide-spread use of this method.

In this context, the recommended article by Muenker *et al.* stands out: it introduces a new way to passively quantify out-of-equilibrium properties of a tracer bead embedded in the intracellular medium that both convincingly relates to important biophysical quantities, and

hints at novel structures for non-equilibrium processes. Specifically, they propose **a way to measure fluctuation-dissipation breakdown from purely passive measurements**.

The key innovation is the introduction of a quantity they call the *mean back relaxation* (MBR). While the precise definition is somewhat convoluted, the idea is pretty simple: it measures how much, after a move forward, the bead will move back. More precisely, the MBR is an average *three-points* quantity: along the measured trajectory $x(t)$ of the bead, consider two points over which, in a short time τ, the bead has moved a distance d. This initial jump (from position $x-d$ at time $t=-\tau$ to x at time $t=0$) serves as a *conditioning*: given these two positions, where will the bead go next, after a time t, on average? The quantity $\mathrm{MBR}(t)$ measures this in units of d: MBR=0 indicates that, on average, the bead stays at x where it was last; MBR=1 indicates that it goes back to $x-d$ where it was previously. The title of the article stems from the similarity of this approach with Onsager's regression hypothesis that intrinsic fluctuations and extrinsic perturbations are physically equivalent: by conditioning the average on the initial jump, the MBR rectifies random fluctuations into a fixed perturbation – thus giving passive access to the *response* function of the system.



The MBR (black curve) is the relaxation of the system after a conditioned jump (green symbols), averaged over an ensemble of trajectories (red; one example in blue).

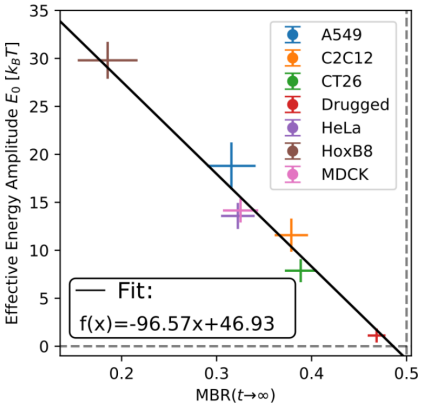
Strikingly, the authors show that for a confined *equilibrium* process, for which detailed balance is observed, we have the following limit:

$$\lim_{t\to\infty} \mathrm{MBR}(t) = \frac{1}{2}$$

which means that *when an equilibrium process moves two steps forward, it will on average go one step back.* This can be proved simply by using the time reversibility of equilibrium processes: regardless of the state of the system (including if there are hidden degrees of freedom), the probability to go, in a time τ, from $x-d$ to x and from x to $x-d$ are equal. Thus, on average, the system eventually relaxes back to the mid-point between x and $x-d$.

In contrast, when the system is driven out-of-equilibrium, this is no longer the case. The authors first explore a minimalist model of the cytoplasm as a nonequilibrium environment: it is modeled as a diffusing trap, moving around and dragging the bead along with it*. This drive results in long-time MBR limit that is $< \frac{1}{2}$: because the bead lags behind, it tends to relax closer to the final than the initial point – with very strong drive it actually keeps going forward, and MBR< 0. By performing *in vitro* experiments where the diffusing cage is realized by optical tweezers, the authors show that this method can be applied in practice. This simple quantity thus captures nonequilibrium coupling between the observed position of the bead, and the hidden position of the diffusing trap – all in the absence of observable currents. To my knowledge, no pre-existing method could convincingly do that for continuous systems.

*Note that this toy model is not confined, which somewhat complicates the interpretation of the result.



The long-term MBR correlates very strongly with the magnitude of FDR breakdown, along many cell types.

The authors do not stop there: they turn to use their newly-defined quantity on experimental data of tracer trajectories in a range of cell types, and find that indeed, the long-time MBR is $< \frac{1}{2}$. They are also able to compare these results with the outcome of traditional FDR breakdown measurements, obtained by a combination of active and passive microrheology. Strikingly – and somewhat mysteriously – the magnitude of FDR breakdown, here characterized as an effective energy E_0, shows a near-perfect linear relation with the MBR. Furthermore, exploiting the time-dependent MBR and some modeling and calibration, the authors are also able to reconstruct the viscoelastic properties of the medium. These intriguing observations show that the simple, passive measurement of the MBR essentially contains the same information as the painstaking FDR breakdown analysis – a fact that will certainly open new avenues for the characterization of the active intracellular medium.

In my opinion, this article is a first step into exploring how 3-point analysis of experimental data can be leveraged into deep insights about nonequilibrium stochastic systems. While the precise way the MBR is defined is probably not optimal, as it requires large amounts of data and appears sensitive to measurement noise, these results are already very promising. We should definitely explore this more – who knows where this might lead?

I thank Timo Betz and Matthias Krüger for clarifying and stimulating discussions. Figures are reproduced from the recommended article with permission of the authors. I acknowledge support by France 2030, the French National Research Agency (ANR-16-CONV-0001), and the Excellence Initiative of Aix-Marseille University – A*MIDEX.

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