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New and Notable

Cell migration: Beyond Brownian motion

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Cell migration is vital, fulfilling essential biological tasks like morphogenesis, wound healing, or the killing of pathogens in organisms. But cell migration also drives detrimental processes like tumor metastasis or inflammation reactions. To understand cell migration by classifying different types of cells defines a fundamental problem of cell science. Recording under a microscope the paths of single cells reveals random-looking migration (see Fig. 1(a), right) that, in terms of physics, reminds of the diffusive Brownian motion of a tracer particle in a fluid. This tracer motion can be described by the Langevin equation (1), a stochastic version of Newton's second law, as it models the random collisions of a tracer with the fluid molecules by a random force. Around the same time as Langevin published his famous equation, Pearson proposed to model the movements of organisms by random walks (2). This idea stipulates that an organism moves in a random direction over a certain distance per time step. These two approaches coined a paradigm that persisted for almost a century, suggesting that biological movements are in general 'so random' that they can be modelled by stochastic processes *without memory*. Pearson conveyed this idea by the picture of a drunken sailor, which can be found in many textbooks of statistical mechanics. That is, an agent makes the next movement step by not remembering the previous one. In mathematical terms, this complete loss of memory is called a *Markov property*.

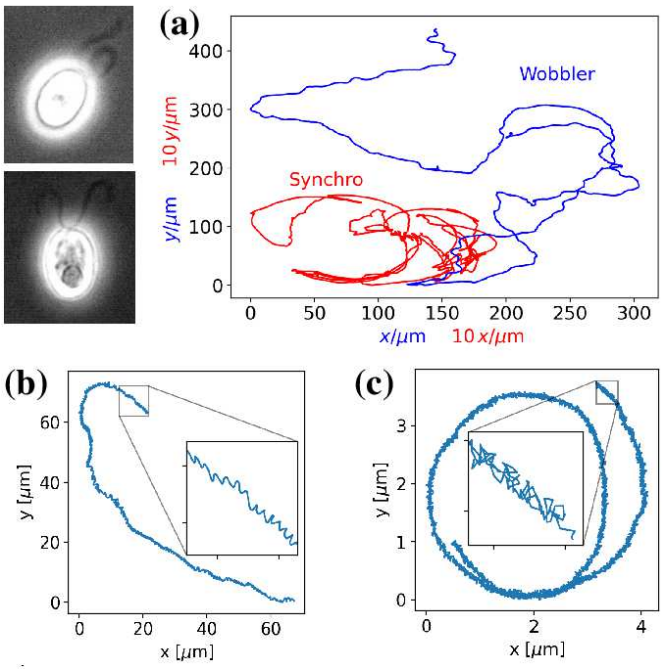

Figure 1: (a) Phase-contrast microscopy images and swimming paths of *Chlamydomonas reinhardtii* algae. These cells exhibit two different swimming modes depending on their diameter, characterised by either wobbler (top image) or synchro-type (bottom) flagellar motion. Shown to the right of (a) are sample paths up to a minute, which on these scales look quite 'random'. (b), (c) Higher resolution data up to a few seconds, plus blow-ups, revealing a non-trivial complex fine structure of the swimming paths for (b) wobbler and (c) synchro (adapted from (3), with kind permission by the authors).

Analysing experimentally recorded paths of different types of migrating cells, decades ago it was reported that cells move according to the predictions of random walks (4), respectively Langevin dynamics (5). Subsequent work, however, challenged

this simple picture by revealing the existence of memory in terms of non-Markovian correlations between different time steps (6). Especially over the past two decades, many articles appeared suggesting that cell migration is typically way more complex than Markovian random motion (7–10). There are indeed many physical and biological reasons to doubt a simplifying description of cell migration patterns by Brownian motion. First of all, a Brownian tracer in a fluid diffuses *passively*, driven by the collisions with the surrounding particles, while a cell moves *actively* by itself converting chemical into kinetic energy (11). Active biological motion, in turn, involves complex microscopic processes, like cytoskeletal fluctuations driving cell crawling, or the beating of flagella steering microswimmers. On top of this, there are interactions with the environment via surface adhesion and biochemical cell-signalling cascades. *Per se* there is no reason why all these complex processes across different spatio-temporal scales should integrate out to yield purely Markovian dynamics. However, assessing non-Markovian correlations of cell migration in experiments for constructing data-driven mathematical models reproducing memory is a notoriously difficult task. It necessitates to compute correlation functions, related to what statisticians call covariances, which are not easy to reliably extract from data, nor to analyse.

In their new contribution to *Biophys.J.* (3) Klimek et al. tackle this problem by analysing experimental trajectories of unicellular *Chlamydomonas reinhardtii* microalgae. These are microswimmers generating different movement paths, depending on whether the flagella driving their motion move synchronously or not, see Fig. 1(b),(c). Using a specific experimental set-up by which they constrain the microswimmers between two glass plates so that they do not move out of focus of the microscope, they generated 3000 position data points for 59 algae each. This data is analysed by using a novel form of generalised Langevin dynamics incorporating non-Markovian memory, thus significantly going beyond Brownian motion.

Very interestingly, the key feature of their theory, the generalised Langevin equation (GLE), can be derived from first principles. Let us assume that all particles forming a system interact according to Newton's classical mechanical equations of motion. Let us identify one of them, with a bigger mass than the others, as a 'walker' while the others act as a 'driving' molecular bath. It is well-known that for this setting, applying a (Mori-Zwanzig) projection operator formalism by making simplifying assumptions, in particular assuming complete loss of memory, the Markovian Langevin equation modelling Brownian motion can be derived. However, this approach can be generalised by including memory in the dynamics leading to non-Markovian GLEs (12). Very recently, Netz and colleagues have worked out a new, more detailed theory along these lines leading to a novel type of GLE (13). This theory has already been applied to reveal non-Markovian effects in protein folding (14) and human breast cancer cell migration (10). Reference (3) continues this line of research by providing compelling evidence that their framework can be used to construct a non-Markovian GLE from data for modelling the motion of *C. reinhardtii* microswimmers. Combining this method with an unbiased cluster analysis they show that, without any prior knowledge, different types of cells can be distinguished by purely analysing their migration patterns. Their numerical findings perfectly match to the different types of cells identified by visual inspection, cf. the different paths in Fig. 1 (b),(c). This paves the way for devising a general method to classify different cells based on motion trajectories only.

A fundamental open question is the generality, or perhaps even universality, of both their underlying theory and their approach for experimental data analysis. One may challenge this framework along three lines: First, the GLE devised by Netz et al. is by far not the only approach to model non-Markovian stochastic dynamics. Apart from many other types of GLEs, like fractional, scaled or superstatistical Brownian motion, there is the very different class of sub- or superdiffusive continuous time random walks (15, 16). The Gaussian noise generating Brownian motion-type dynamics may furthermore be replaced by power laws yielding Lévy processes (17). The virtue of the theory by Netz et al. is that, in contrast to all these other stochastic processes, it has a sound physical foundation in terms of classical mechanics. On the other hand, one may wonder whether all physical and biological systems, no matter how complex they are, need to be understood starting from microscopic Hamiltonian many-particle dynamics. Furthermore, non-Markovian dynamics driven by power laws exhibits intricate phenomena like non-stationarity, ageing, weak ergodicity breaking and long-term anomalous diffusion, all well observed in physical and biological experiments (15, 16). To what extent the theoretical framework put forward by Netz et al. can cover these properties as well remains an interesting open question.

Second, this theory relies on the important assumption of Gaussianity (normality) of relevant probability distributions by exploiting fluctuation-dissipation relations (FDRs). But the former excludes Lévy walks that attracted considerable attention over the past two decades for modelling organismic movement (17), including cell migration (9). Since Lévy distributions obey a generalised central limit theorem (15), there is a reason why one should expect to see them in nature. Another interesting point is that for describing *C. reinhardtii* microswimming the authors map a non-Markovian GLE without FDR onto an effective GLE with FDR. But this again relies on Gaussianity. Whether this method can be extended to non-Gaussian dynamics, and to non-equilibrium processes like chemotaxis, poses further inspiring open questions (18).

Third, the validity of FDRs is intimately related to distinguishing active from passive dynamics. As explained at the beginnng of this article, the original Langevin equation models the passive motion of a Brownian tracer. This follows from its physical derivation based on microscopic Hamiltonian dynamics yielding diffusion in non-living matter. However, within the past two decades the new concept of active matter was developed to understand biological processes in living systems,

like the formation of tissues, swarming, or crowd dynamics. A special case within this framework is the theory of active Brownian particles, modelling the movements of small agents like bacteria, daphnia, or artificial Janus particles (11, 19). All active Brownian particle models feature broken FDRs, mathematically expressing the externally driven activity of biological dynamics. On the other hand, they have all been formulated phenomenologically, instead of being derived from Hamiltonian dynamics, like GLEs exhibiting FDRs. To what extent standard models of active Brownian motion (11, 19) can be derived from first principles forms yet another crucial open question. Recently, the dynamics of a passive tracer in an active bath of microswimmers has been calculated from microscopic non-Hamiltonian equations of motion (20), taking special care of the hydrodynamic interaction between tracer and bath. Combining this with other techniques for constructing a biomolecular model of cell migration poses a formidable challenge for future research.

DECLARATION OF INTERESTS

The author declares no competing interests.

REFERENCES

1. Langevin, P., 1908. Sur la théorie du mouvement brownien. *C.R. Acad. Sci. (Paris)* 146:530–533. See also the English translation in Am. J. Phys. **65**, 1079 (1997).

2. Pearson, K., 1906. Mathematical contributions to the theory of evolution - A mathematical theory of random migration. *Biometric ser.* 3:54.

3. Klimek, A., D. Mondal, S. Block, P. Sharma, and R. R. Netz, 2024. Data-driven classification of individual cells by their non-Markovian motion. *Biophys. J.* 123:1173–1183

4. Gail, M., and C. Boone, 1970. The locomotion of mouse fibroblasts in tissue culture. *Biophys. J.* 10:980–993.

5. Dunn, G., and A. Brown, 1987. A unified approach to analysing cell motility. *J. Cell Sci. Suppl.* 8:81–102.

6. Hartmann, R., K. Lau, W. Chou, and T. Coates, 1994. The fundamental motor of the human neutrophil is not random: Evidence for local non-Markov movement in neutrophils. *Biophys. J.* 67:2535–2545.

7. Rieu, J., A. Upadhyaya, J. Glazier, N. Ouchi, and Y. Sawada, 2000. Diffusion and deformations of single Hydra cells in cellular aggregates. *Biophys. J.* 79:1903–1914.

8. Dieterich, P., R. Klages, R. Preuss, and A. Schwab, 2008. Anomalous dynamics of cell migration. *PNAS* 105:459–463.

9. Harris, T., E. Banigan, D. Christian, et al., 2012. Generalized Lévy walks and the role of chemokines in migration of effector CD8+ T cells. *Nature* 486:545–548.

10. Mitterwallner, B. G., C. Schreiber, J. O. Daldrop, J. O. Rädler, and R. R. Netz, 2020. Non-Markovian data-driven modeling of single-cell motility. *Phys. Rev. E* 101:032408.

11. Romanczuk, P., M. Bär, W. Ebeling, B. Lindner, and L. Schimansky-Geier, 2012. Active Brownian particles. *Eur. Phys. J. Spec. Top.* 202:1–162.

12. Zwanzig, R., 2001. Nonequilibrium statistical mechanics. Oxford University Press, Oxford.

13. Ayaz, C., L. Scalfi, B. A. Dalton, and R. R. Netz, 2022. Generalized Langevin equation with a nonlinear potential of mean force and nonlinear memory friction from a hybrid projection scheme. *Phys. Rev. E* 105:054138.

14. Dalton, B. A., C. Ayaz, H. Kiefer, A. Klimek, L. Tepper, and R. R. Netz, 2023. Fast protein folding is governed by memory-dependent friction. *PNAS* 120:e2220068120.

15. Klages, R., G. Radons, and I. M. Sokolov, editors, 2008. Anomalous transport: Foundations and Applications. Wiley-VCH, Berlin.

16. Metzler, R., J.-H. Jeon, A. G. Cherstvy, and E. Barkai, 2014. Anomalous diffusion models and their properties: non-stationarity, non-ergodicity, and ageing at the centenary of single particle tracking. *Phys. Chem. Chem. Phys.* 16:24128–24164.

17. Viswanathan, G., M. da Luz, E. Raposo, and H. Stanley, 2011. The Physics of Foraging. Cambridge University Press, Cambridge.

18. Netz, R., 2023. Derivation of the non-equilibrium generalized Langevin equation from a generic time-dependent Hamiltonian. *preprint arXiv:2310.00748* .

19. Bechinger, C., R. Di Leonardo, H. Löwen, C. Reichhardt, G. Volpe, and G. Volpe, 2016. Active particles in complex and crowded environments. *Rev. Mod. Phys.* 88:045006.

20. Kanazawa, K., T. G. Sano, A. Cairoli, and A. Baule, 2020. Loopy Lévy flights enhance tracer diffusion in active suspensions. *Nature* 120:364–367.