

Reply to: Insufficient evidence for ageing in protein dynamics

Jun Li¹, Xiaohu Hu², Thomas Neusius³, Xiaolin Cheng⁴, Micholas Dean Smith^{5,6}, Aljaž Godec⁷, Liang Hong¹, Ralf Metzler⁸ and Jeremy C. Smith^{5,6,*}

¹School of Physics and Astronomy and Institute of Natural Sciences, Shanghai Jiao Tong University, Shanghai, China.

²Schroedinger Inc., New York, NY, USA.

³Wiesbaden Business School, RheinMain University of Applied Sciences, Wiesbaden, Germany.

⁴Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, OH, USA.

⁵UT/ORNL Center for Molecular Biophysics, Oak Ridge National Laboratory, Oak Ridge, TN, USA.

⁶Department of Biochemistry and Cellular and Molecular Biology, University of Tennessee, Knoxville, TN, USA.

⁷Mathematical bioPhysics Group, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany. ⁸Institute of Physics & Astronomy, University of Potsdam, Potsdam, Germany.

*e-mail: smithjc@ornl.gov

Replying to: I. Goychuk & T. Pöschel *Nature Physics* <https://doi.org/10.1038/s41567-021-01269-1> (2021)

Reference 1 states that we² claimed that internal motions in single protein molecules exhibit ageing over 13 decades of time. In contrast, our primary conclusion, as expressed in the title, was that over these timescales the dynamics of single protein molecules is non-equilibrium and self-similar². Notwithstanding, we demonstrate here that protein dynamics does indeed exhibit ageing according to the criteria of ref. 1.

Reference 1 claims that to adequately describe the ageing process a two-time point correlation function should be calculated. In Fig. 1a, we plot the following two-time correlation function using the $T = 17\text{-}\mu\text{s}$ -simulation-length trajectory from ref. 2 of the protein phosphoglycerate kinase (PGK):

$$K(t_{\text{ag}}, t_{\text{ag}} + \Delta; T) = \frac{1}{T - t_{\text{ag}} - \Delta} \int_0^{T - t_{\text{ag}} - \Delta} \delta R(t_{\text{ag}} + t') \delta R(t_{\text{ag}} + t' + \Delta) dt' \quad (1)$$

where Δ is the lag time, t is the time, t_{ag} is the ageing time and δR is the deviation of the interdomain distance of PGK from its time-averaged value. In the corresponding equation (3) of ref. 1, R is used instead of δR , which renders the interdomain correlation function meaningless as no appreciable decay is present. Furthermore, the literature cited in ref. 1 (ref. 3) also defines wide-sense stationarity (that is, in the mean-square sense) in terms of δR .

As shown in Fig. 1a, $K(t_{\text{ag}}, t_{\text{ag}} + \Delta; T)$ shifts upon changing t_{ag} , hence, according to ref. 1, demonstrating ageing. Further confirmation of ageing was obtained by carrying out 100 independent molecular dynamics simulations of PGK, each of $T = 100$ ns, and plotting the ensemble- and time-averaged mean square atomic displacement, $\langle \overline{\delta^2(\Delta, T)} \rangle$, as a function of t (that is, $T - t_{\text{ag}}$) at fixed Δ (Fig. 1b). An appreciable decay of $\langle \overline{\delta^2(\Delta, T)} \rangle$ with t is observed, and is direct evidence of ageing.^{4,5}

In Fig. 2a of ref. 2 the time-averaged (not ensemble-averaged) mean square atomic displacement, $\overline{\delta^2(\Delta, T)}$ is shown for different t . Reference 1 states that Fig. 2a of ref. 2 was used to claim ageing and the absence of ergodicity. However, neither ageing nor non-ergodicity were claimed from this plot. Instead, the conclusion from Fig. 2a–c of ref. 2 was the presence of strong non-stationarity. $\Delta \ll t$ is stipulated in ref. 1 as a condition for statistical validity. In Fig. 2a of ref. 2 the 10 ns simulation line deviates from that of the 100 ps simulation for $\Delta > 1$ ps (and not $\Delta > 30$ ps as stated in ref. 1). Further, $\overline{\delta^2(\Delta, T)}$ from the 17 μ s simulation deviates from that of 500 ns for $\Delta > 100$ ps. Therefore, the t/Δ are $\sim 10^2$ and $\sim 10^4$, respectively, satisfying the requirement of ref. 1. Also, an overlap of $\overline{\delta^2(\Delta, T)}$ at different t at small Δ , as seen in Fig. 2a of ref. 2, arising from stationary noise, exists for a confined noisy continuous-time random walk,^{5,6} which is the non-ergodic model used to interpret the simulation data in ref. 2.

Figure 1 here, as well as other pieces of evidence in ref. 2, satisfy the conditions for ageing claimed in ref. 1. However, ref. 4 shows that, even if two-time correlation functions such as equation (1) do exhibit non-stationarity, this does not prove breaking of time translational invariance. Indeed, according to equation (1) (and equation (3) in ref. 1) even ergodic dynamics, if evolving from a non-stationary initial condition, exhibits transient ageing⁴. Using rigorous mathematics ref. 4 instead shows that the dynamics of PGK does indeed exhibit breaking of time translational invariance. Moreover, ref. 4 shows that all systems tracking low-dimensional observables projected from the full system phase space as a function of time for which (1) the projection renders the reduced dynamics non-Markovian and (2) the observable is not prepared in, or initially sampled from and averaged over, a stationary distribution always exhibit non-equilibrium dynamic time asymmetry (DTA). DTA sports characteristics commonly associated with ageing. However, DTA and the definition of ageing adopted by the theoretical glass physics community are distinguishable: the latter requires that the relaxation time be beyond the longest times accessible to experiment or simulation, which itself typically requires that the relaxation time increase exponentially with system size.⁷⁻¹¹

In contrast to the claims in ref. 1, the results in ref. 2, together with the above analysis, the analysis in ref. 4 and experimental results from single-molecule spectroscopic time series published over the intervening 5 yr (refs. 12,13) since ref. 2 was published, have led to the unequivocal conclusion that the internal motions of a single protein are non-equilibrium, self-similar and, according to the criteria of ref. 1, both non-ergodic and ageing. More generally, DTA is ubiquitous in complex materials and biological systems, and must surely change the way in which many experiments and simulations are interpreted.

References

1. Goychuk, I. & Pöschel, T. Insufficient evidence for ageing in protein dynamics. *Nat. Phys.* <https://doi.org/10.1038/s41567-021-01269-1> (2021).
2. Hu, X. et al. The dynamics of single protein molecules is non-equilibrium and self-similar over thirteen decades in time. *Nat. Phys.* <https://doi.org/10.1038/nphys3553> (2016).
3. Papoulis, A. & Saunders, H. *Probability, Random Variables and Stochastic Processes* (McGraw-Hill, 1989).
4. Lapolla, A., Smith, J. C. & Godec, A. Ubiquitous dynamical time asymmetry in measurements on materials and biological systems. Preprint at <https://arxiv.org/abs/2102.01666> (2021).
5. Jeon, J. H., Barkai, E. & Metzler, R. Noisy continuous time random walks. *J. Chem. Phys.* **139**, 121916 (2013).
6. Neusius, T., Sokolov, I. M. & Smith, J. C. Subdiffusion in time-averaged, confined random walks. *Phys. Rev. E* **80**, 011109 (2009).
7. Bouchaud, J.-P. & Dean, D. S. Aging on Parisi's tree. *J. Phys. I* **5**, 265–286 (1995).
8. Monthus, C. & Bouchaud, J.-P. Models of traps and glass phenomenology. *J. Phys. A* **29**, 3847–3869 (1996).
9. Ritort, F. & Sollich, P. Glassy dynamics of kinetically constrained models. *Adv. Phys.* **52**, 219–342 (2003).
10. Bovier, A. & Faggionato, A. Spectral characterization of aging: the REM-like trap model. *Ann. Appl. Probab.* **15**, 1997–2037 (2005).
11. Vincent, E., Hammann, J., Ocio, M., Bouchaud, J.-P. & Cugliandolo, L. F. Slow dynamics and aging in spin glasses. *Lect. Notes Phys.* **492**, 184–219 (1997).
12. Morgan, I. L., Avinery, R., Rahamim, G., Beck, R. & Saleh, O. A. Glassy dynamics and memory effects in an intrinsically disordered protein construct. *Phys. Rev. Lett.* **125**, 058001 (2020).
13. Ye, W. et al. Conformational dynamics of a single protein monitored for 24 h at video rate. *Nano Lett.* **18**, 6633–6637 (2018).

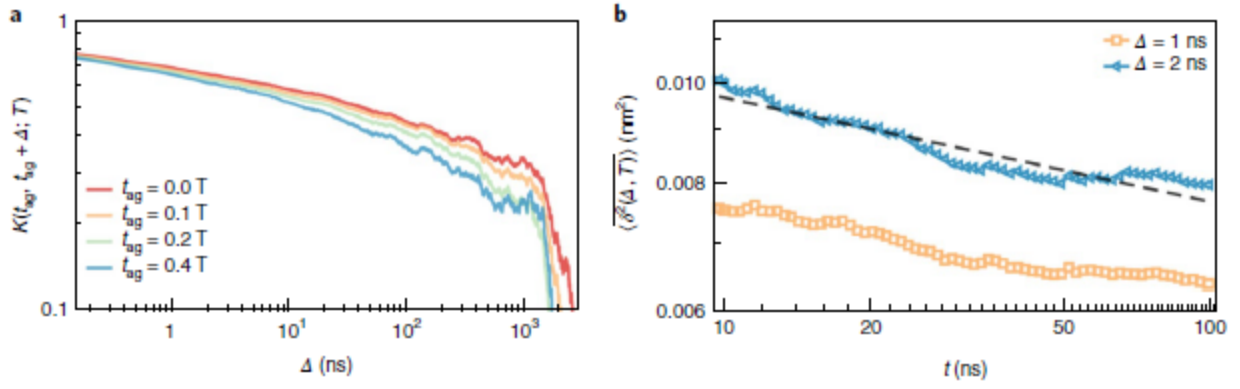


Figure 1. Demonstration of ageing in molecular dynamics of PGK. **a**, Two-time autocorrelation function defined in equation (1). **b**, Ensemble- and time-averaged mean square displacement as a function of t at fixed Δ .