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Insufficient evidence for ageing in protein dynamics

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In their Letter, Hu et al.[1] claimed that the non-equilibrium dynamics of single protein molecules exhibits ageing over 13 decades of time, which covers the duration of the lifetime of many proteins. The Letter was the subject of a News and Views article[2], and continues to attract the attention of many researchers. Here we re-examine the foundation of this claim and show that it is based on a fallacy.

The numerical results shown in Fig. 2a of ref. [1] are obtained from Supplementary equation (1) in the same paper:

$$\overline{\delta^2(\Delta;t)} = \frac{1}{t-\Delta}\int_0^{t-\Delta} [\delta(\Delta;t')]^2\, \mathrm{d}t' \qquad (1)$$

where $\delta(\Delta;t) = R(t+\Delta) - R(t)$ is an increment of the random process $R(t)$ defining the distance between two points in the protein within the time interval $(t, t+\Delta)$, Δ is the lag time and t is the time. Equation (1) can be understood as the time average of the random process $A_\Delta(t) \equiv \delta^2(\Delta;t)$ at fixed Δ within the time window $\mathcal{T} = t - \Delta$ (refs. [3,4]). The ensemble average, $\langle A_\Delta(t)\rangle = (1/N)\sum_{i=1}^N A_\Delta^{(i)}(t)$ as $N \to \infty$ agrees with the time average for $\mathcal{T} = t - \Delta \to \infty$ provided that the random process is ergodic. Thus, to replace the time average by the ensemble average or vice versa, we have to check for ergodicity. A random process $A_\Delta(t)$ is ergodic in the mean value if and only if it is stationary and the time average of the stationary ensemble-averaged autocorrelation (or autocovariance) function of $A_\Delta(t)$ decays asymptotically to zero[3].

In any experiment, $\Delta \ll \mathcal{T}$ must be satisfied[3,4]. Otherwise, the time average makes little statistical sense and remains a highly fluctuating random variable, similar to an ensemble average for small N. In light of these arguments, we now examine the conclusions drawn from the data presented by Hu et al.

From their Fig. 2a, the authors infer ageing from the monotonic decrease of the slope of the time average $\overline{\delta^2(\Delta;t)}$ as Δ and the observation time t both increase. We do not believe that this is a valid argument. Instead, we interpret the same data in a different way. In the limit $t \gg \Delta$, fluctuations of $\overline{\delta^2(\Delta;t)} = \overline{A_\Delta(t)}$ vanish for ergodic processes[3]. Therefore, for any measurement with fixed Δ, the value of the time average $\overline{A_\Delta(t)}$ should not significantly depend on t, provided $t \gg \Delta$.

Let us consider Fig. 2a in ref. [1] for $t = 100$ ps and $t = 10$ ns. The curves $\overline{\delta^2(\Delta;t)}$ agree perfectly for a large range of Δ, except for $\Delta \gtrsim 30$ ps, where the data deviate due to the fact that the condition $t = 100\,\text{ps} \gg 30\,\text{ps} = \Delta$ is violated. Almost perfect agreement is also found for $t = 10$ ns and $t = 500$ ns, in the entire range of overlapping $\Delta \in (10^1, 10^3)$ ps, and the same is true for $t = 500$ ns and $t = 17$ µs. Only in the last combination is the range of agreement smaller, which may be attributed to the different averaging procedure[1] used for $t = 17$ µs. From the near overlap of the data, which in some cases is closer than the line width, we conclude that there is no reason to claim ageing, or absence of ergodicity. The observation that $\overline{\delta^2(\Delta;t)}$ does not saturate with increasing Δ is not evidence for ageing either.

Similarly, the autocorrelation function, $C(\Delta;t)$, presented in Fig. 2b of ref. [1] is interpreted incorrectly. The data shown are the normalized C' calculated according to Supplementary equation (4) in ref. [1]

$$C'(\Delta;t) = \frac{1}{t-\Delta}\int_0^{t-\Delta} \delta R(t')\,\delta R(t'+\Delta)\,\mathrm{d}t' \qquad (2)$$

with $\delta R(t) = R(t) - \langle R\rangle$, where $\langle \ldots \rangle$ denotes the ensemble average. If $R(t)$ is ergodic, in the limit $t \to \infty$, this is an accurate definition of the time-averaged stationary autocorrelation function[3]. If, however, $R(t)$ is a non-ergodic process, as claimed by the authors, the ensemble average $\langle R\rangle$ cannot replace the time average $\overline{R(t;t^*)}$, within a certain time interval $t^* \to \infty$. Yet this substitution was made by Hu et al. in the definition of the trajectory's fluctuation, $\delta R(t)$.

For an adequate description of the ageing process, the autocorrelation function cannot depend only on the time difference Δ (refs. [5–7]). Instead, it would require a two-time autocorrelation function[3]

$$K(t_{ag}, t_{ag}+\Delta;t) = \frac{1}{t-\Delta}\int_0^{t-\Delta} R(t_{ag}+t')R(t_{ag}+t'+\Delta)\,\mathrm{d}t'. \qquad (3)$$

Here we omit time averages of $R(t)$ for clarity, meaning that it is not an autocovariance function. This autocorrelation function is a trajectory-averaged counterpart of the ensemble-averaged $\langle R(t_{ag})R(t_{ag}+\Delta)\rangle$, where t_{ag} is the ageing time[5–7].

We believe that the conclusions drawn by Hu et al. are consequences of an insufficient mathematical formalism for the computation of the autocorrelation function. In particular, Hu et al. confuse the time-averaging window, t, in the stationary autocorrelation function, $C(\Delta;t)$, shown in Fig. 2b of ref. [1], with the ageing time (our t_{ag}) in the two-time non-stationary autocorrelation function. The steep decay of the autocorrelation function for $\Delta \gtrsim \tau_c$ defined by crossing the level 1/e in Fig. 2b of ref. [1] is an artefact resulting from the strong dependence of $C'(\Delta;t)$ on Δ/t in equation (2) beyond its range of validity, $t \gg \Delta$, when Δ approaches t, and $\mathcal{T}$ tends to zero.

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Competing interests
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