exactly the same extent. To explain, because each cause of death plays out along a distinct timescale, if a particular condition were to decrease the odds of a fast-acting cause of death but not a slow-acting cause (or vice versa), a particular region of the overall survival curve would be altered, and thus its overall shape changed (Fig. 1b). Even interventions such as temperature shifts might be expected to affect different causes of death differently. After all, death is a biochemical process, and changing temperatures will alter the rates of different death-promoting chemical reactions differently, depending on the activation energies of those reactions. The authors' observation of almost-perfect timescaling across different conditions thus places specific constraints on how these conditions influence survival.

How can this surprising observation be explained? One possibility is that every cause of death in the worms has the same activation energy and responds identically to changes in food source, toxic exposures and diverse genetic mutations. Another is that worms have a single mechanistic cause of death.

A more plausible interpretation is that there is some intermediate state on which all the tested interventions converge, and which determines the risk of death from each possible cause (Fig. 1c). Could this intermediate state involve, for instance, the insulin/insulin-like growth factor signalling (IIS) pathway, which is central to many aspects of ageing across species[3]? No — the authors found that survival curves retain their shape even when the IIS pathway is inactivated, and in response to conditions and mutations known to act independently of the IIS pathway.

The most likely explanation for this intermediate state is that the risk of death is governed not by any single pathway, but by a property that arises from interactions between the various molecular processes that influence ageing. This property, perhaps best called 'resilience', would be an intrinsic biological property of ageing *C. elegans*, just as temperature and pressure are intrinsic thermodynamic properties of gases that emerge from the interactions of the constituent molecules.

The temperature of water in a whistling tea kettle provides an analogy for resilience. There are many ways in which to heat the water — on a stove, in a microwave, or even by adding a strong acid. However, whether a kettle whistles depends not on the source of the heat, but on the water temperature. Similarly, alterations in the molecular processes that contribute to resilience could change the rate of ageing (the heating rate of the water) without changing its underlying nature (the relationship between temperature and whistling).

The authors use detailed simulations to demonstrate how such a property could emerge. If resilience is a measure of the fraction of biological processes in a densely interconnected network that have failed, then manipulations that alter a subset of these processes will extend or shorten lifespan without changing the shape of the survival curve. Alternatively, a single physical property, which is acted on by many molecular processes and affects the risk of death from diverse causes, could underlie an organism's resilience. Potential candidates for such physical properties include intracellular redox levels[4,5] or global protein solubility levels and turnover rates[6,7]. Whatever the case, the current work provides a strong constraint on any proposed molecular mechanism of resilience — measurements of the levels or activity of that mechanism must correlate exactly with lifespan across temperatures and among different genetic mutants.

This study suggests that concepts such as resilience and frailty, long used in the ageing literature, might have a concrete biological meaning. In particular, the Rockwood frailty index, which calculates the fraction of measured clinical markers considered to be in a deficient state[8], is a close theoretical match for the authors' interpretation of resilience. Most importantly, these results demonstrate that, although students of ageing biology have learnt much about how to manipulate the rate of ageing, the nature of organismal frailty is almost completely unknown. The few interventions the authors identify that do change the shape of the survival curve in *C. elegans* (such as a mutation that alters feeding ability, and another that alters function in mitochondria, the cell's energy centres) may point the way towards understanding this previously unappreciated biology.

Finally, although Stroustrup *et al.* consider only lifespan, increasing chronological lifespan does not necessarily increase the fraction of lifespan spent in good health[9]. Further work of a similar experimental and analytical rigour will be necessary to clarify the relationship between quality and quantity of life. ■

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PARKINSON'S DISEASE

# Disorder in the court

**The native structure of the protein α-synuclein, which is implicated in Parkinson's disease, is controversial. In-cell nuclear magnetic resonance now shows that it remains disordered when loaded into living cells.** SEE ARTICLE P.45

T. REID ALDERSON & AD BAX

The 3D structure of a biological molecule typically dictates its function. In an apparent paradox, intrinsically disordered proteins (IDPs) lack well-defined 3D structures, but this structural plasticity can confer diverse biological functions. The small protein α-synuclein is an IDP *in vitro*, but whether it exists *in vivo* as a monomeric IDP[1] or as an ordered, helical tetramer[2,3] is a matter of dispute[4]. If α-synuclein is an ordered tetramer *in vivo*, it might be possible to design drugs that stabilize this protein state and prevent the protein from forming toxic aggregates, which are associated with Parkinson's disease. On page 45 of this issue, Theillet *et al.*[5] describe a detailed analysis of the structure of α-synuclein inside living cells.

Ever since the structure of DNA was unveiled in 1953, the importance of understanding biology at the atomic level has been widely appreciated. But the standard method for resolving structures at atomic resolution, X-ray diffraction, relies on the generation of carefully grown crystals, which involves separating molecules of interest from their native environments. This process can bias the structures of molecules in ways that are hard to predict.

An alternative to X-ray diffraction is nuclear magnetic resonance (NMR) spectroscopy, which can be used to analyse molecules under the same conditions of pH, temperature and ionic concentration as when they are inside cells. In the past decade, it has become possible to use NMR spectroscopy to determine the structure and dynamics of proteins in living cells at atomic resolution[6,7]. In these in-cell studies, enrichment of molecules

of interest with the stable, non-perturbing isotopes nitrogen-15 or carbon-13 permits the NMR signals of these molecules to be filtered from the sea of other cellular components. In 2009, in-cell NMR was used to determine the first structure of a protein inside live bacteria[6], but loading proteins of interest into mammalian cells in large-enough quantities and keeping these cells alive for long enough to perform NMR has been a challenge. Theillet *et al.* have overcome these obstacles, and have used in-cell NMR and a related technique, electron paramagnetic resonance (EPR) spectroscopy, to characterize the structure and dynamics of α-synuclein inside mammalian cells.

α-Synuclein is abundant in many cell types, particularly neurons, where it is found in structures called presynaptic termini, which make contact with and signal to other cells[8]. The protein has a high affinity for certain types of membrane, and it is thought to modulate the release and recycling of presynaptic vesicles that contain neurotransmitter molecules[9]. Many biophysical studies have concluded that, in its natural state, α-synuclein is an IDP that adopts α-helical conformations when it binds to membranes, but these studies mainly used purification conditions that would have denatured (unfolded the structure of) the protein[4]. Thus, it could be that the protein's disorder in the absence of a membrane is a consequence of purification protocols, rather than an intrinsic property of the protein. By inference, this criticism could equally apply to many other IDPs.

In 2011, it was reported that, rather than being an IDP, α-synuclein exists as a helical tetramer when purified from mammalian cells under non-denaturing conditions[2]. This finding took researchers by surprise and ignited a vigorous debate[1,4]. Proponents of the work argue that α-synuclein forms tetramers in living cells, and suggest that these tetramers had dissociated during purification in previous studies[2,3,10]. They further propose that the tetrameric state, which might require a stabilizing cofactor *in vivo*[10], acts as a storage mechanism that safeguards the protein from forming toxic aggregates[2,3,10]. However, subsequent attempts to confirm the existence of helical tetramers have failed[1,4]. Furthermore, other studies have shown that when α-synuclein is added to *in vitro* samples in its monomeric, disordered state, it can aid the assembly of the presynaptic-vesicle fusion machinery[9,11].

Theillet *et al.* introduced a bacterial form of α-synuclein into five types of mammalian cell at concentrations close to those found *in vivo*. They find compelling evidence that the protein remains highly disordered in these conditions, with each monomer changing shape rapidly. Remarkably, despite the fact that IDPs might be expected to be sensitive to degradation, bacterially produced α-synuclein remained intact for days in the authors' cell lines — two of which are closely related to human neurons. Theillet and colleagues confirmed that the cells were not adversely affected by the α-synuclein. Moreover, in a follow-up report published in *Nature Communications*[12], the same group demonstrated that the cells retained the ability to repair damaged forms of bacterially produced α-synuclein, indicating that the protein can interact functionally with the cells' molecular machinery.

By analysing the in-cell NMR and pulsed EPR spectra from α-synuclein, Theillet and colleagues demonstrated that the signals from the in-cell protein were at similar positions to those of the disordered, *in vitro* reference protein — in particular, NMR signals were sharp and showed that the protein remains highly flexible and undergoes rapid molecular reorientation (Fig. 1). The atomic motions of the internalized α-synuclein were slightly more sluggish than those of the reference protein, presumably because of the viscosity of the cellular environment, and the protein showed transient, localized hydrophobic and electrostatic interactions with components of the cytoplasm. The authors' analysis provides the first insight into how individual residues in an IDP interact with the intracellular environment.

Finally, Theillet *et al.* showed that high intracellular concentrations of macromolecules and other cellular components can cause α-synuclein monomers to become slightly more compact, perhaps protecting the monomers from forming the stable intermolecular interactions seen in the toxic, aggregated form of the protein.

Although this study demonstrates that α-synuclein does not fold into a tetramer when introduced into mammalian cells, the authors' data do not fully exclude the existence of a tetrameric state. NMR signals from a small population of tetramers would be broad (an indication that a structure undergoes molecular reorientation only slowly) and weak (Fig. 1), and would therefore be invisible with in-cell NMR. Thus, the protein could exist in a dynamic equilibrium between monomeric and tetrameric states[3,10]. Up to 20% of α-synuclein could exist in a tetrameric state at any one time, because the NMR signal intensities from the monomer accounted for 90%, plus or minus 10%, of the total amount of introduced protein. Thus, the tetramer hypothesis could potentially be reconciled with Theillet and colleagues' results — but only if the intrinsic tetramer concentration is low, or if the otherwise intact machinery of the cells used in the work somehow lacks the ability to convert monomeric α-synuclein into tetramers. ■

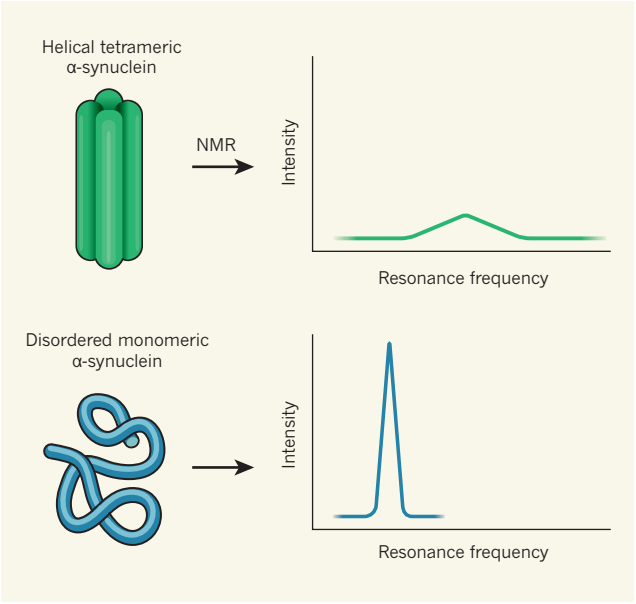


**Figure 1 | Structure of a disordered protein in living cells.** There has been much debate about whether the protein α-synuclein exists as a structured, helical tetramer in healthy cells, or whether it is an intrinisically disordered monomer with no fixed structure. Theillet *et al.*[5] used in-cell nuclear magnetic resonance (NMR) spectroscopy to address this question. If the protein formed a helical tetramer, then NMR spectroscopy would produce broad signals, which are characteristic of structured tetramers undergoing slow molecular reorientation. However, the authors find that the protein reorients rapidly, giving sharp NMR peaks at positions that are indicative of the disordered monomer.

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