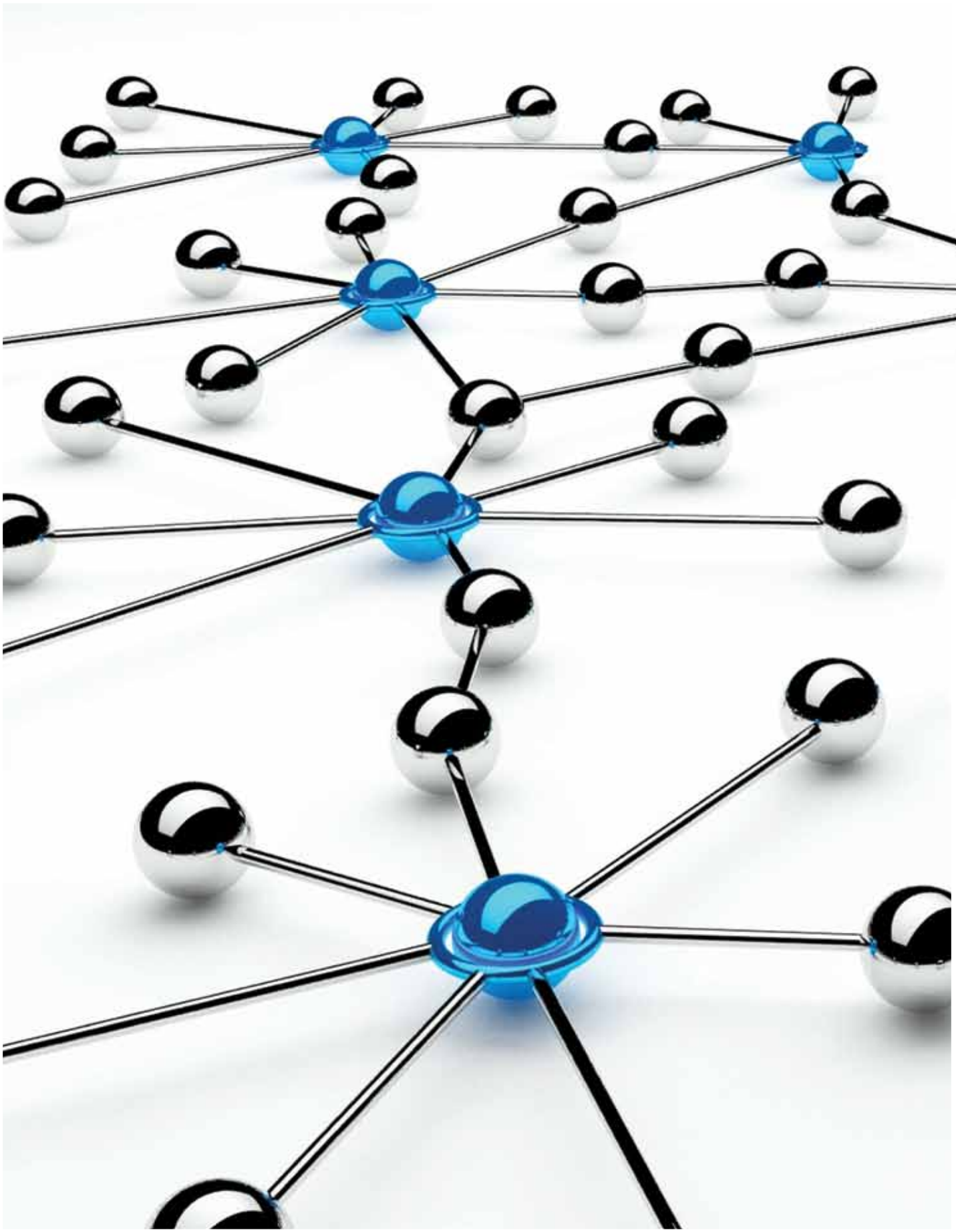
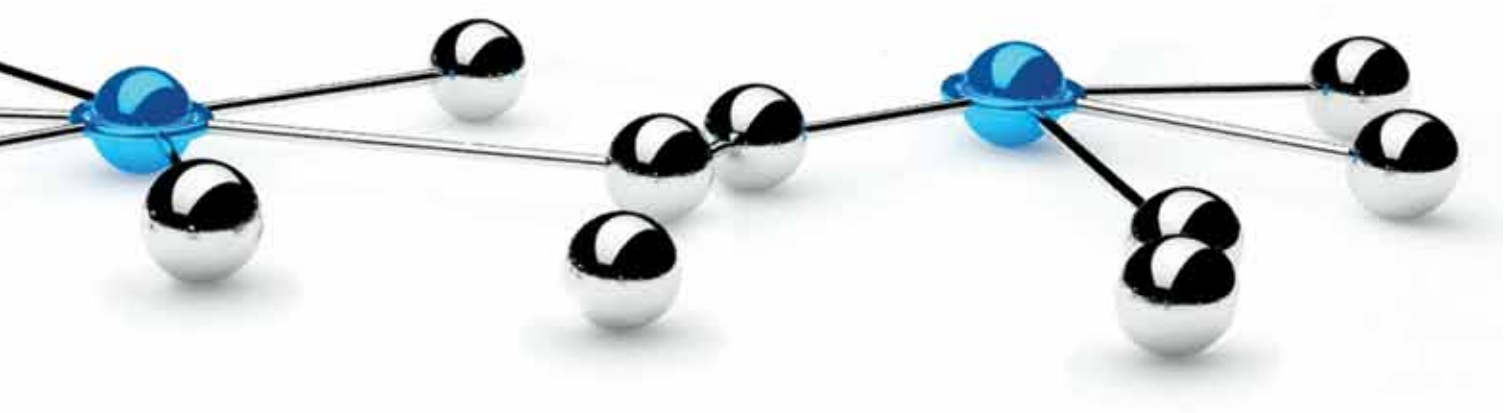


A sticky end?

Rather than evolving to increase complexity, could protein–protein interactions be part of a self-protection strategy gone too far? Philip Ball investigates



During the course of the Human Genome Project to decode the sequence of human DNA, it became clear that we have far fewer genes than previously thought. In 2000 the number was estimated at perhaps 50 000–90 000. The current figure is a little over 20 000. How can organisms so complicated be constructed from so few 'instructions'?

The answer seems in part to be that it's not so much about how many genes you have, but how you use them. Genes act together in complex networks of interactions, with some serving multiple functions depending on which others they interact with. What this often means in practice is that the proteins encoded by the genes stick together to carry out their tasks.

To understand how cells function, we therefore need to decode the so-called interactome, the catalogue of different protein–protein interactions. This network is far more complex in humans and other higher organisms than it is in bacteria and other single-celled prokaryotes. Some bacteria have up to a quarter as many genes as humans, but with far fewer protein interactions.

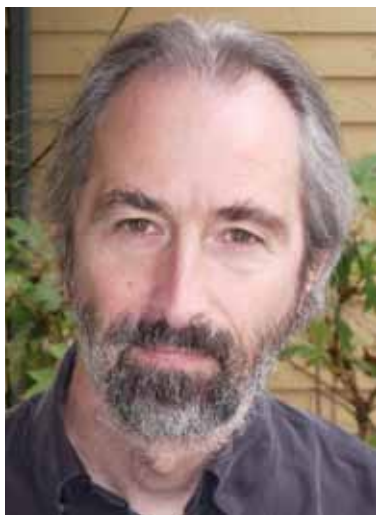
We might imagine that the complexity of the human interactome has accumulated by Darwinian adaptation: that some new protein–protein interactions gave rise to favourable functions, and were therefore selected for. But recent work has challenged that cosy assumption. Biophysicist Ariel Fernández, working at the University of Chicago, US, and now at the Mathematics Institute of Argentina in Buenos Aires, and biologist Michael Lynch of Indiana University in Bloomington, US, claim that the complexity of the human interactome wasn't selected for its adaptive benefits. Rather, it has been forced upon us in an attempt to prevent our proteins from unravelling. According to Fernández and Lynch, it's a consequence of how random mutations in gene sequences tend to make proteins vulnerable to the intrusion of water.[1]

'It is troubling that this molecular machinery is a lot more complex than it would seem to need to be,' says biochemist Nick Lane of University College London, UK. He feels that Fernández and Lynch offer 'pleasing arguments which really have to be at least partly true'.

If they are, this isn't just a startling example of an important aspect of cell biology that is non-adaptive. It's also worrying. For this method of protecting proteins is sure to have its limits, and Fernández thinks that, by covering up the underlying problem, interactome complexity may simply allow it to grow steadily worse. Eventually, he says, our proteins might accumulate so much 'damage' that nothing will prevent them from losing their shape and function. In other words, a short-term solution might just create a time bomb.

In short

- **Complexity in higher organisms is driven by interactions between proteins**
- **Recent work argues that these interactions arise because of random mutations rather than natural selection**
- **These mutations disrupt protein stability but can be covered up by protein–protein interactions**
- **Eventually the accumulated damage could lead to proteins losing their shape and function. Prions and amyloid aggregation might be early signs of this degeneration**



Ariel Fernández and Michael Lynch have proposed that protein–protein interactions are covering up a serious evolutionary problem

Get the drift

The basic problem is that there aren't enough of us. Compared to the swarming hordes of prokaryotic microbes, the six billion people on the planet are a miniscule population. This means we, and other less-numerous eukaryotic organisms, are far more susceptible to a process called random genetic drift, which changes the gene pool in non-adaptive ways.

Natural selection tends to ensure that organisms that are best equipped in genetic terms survive, reproduce and pass on their genes. But this won't always be the case – some organisms will get eaten or catch fatal diseases despite having good genetic fitness, just by bad luck. In huge populations such non-selective effects are small, but chance plays a bigger role if the numbers are smaller.

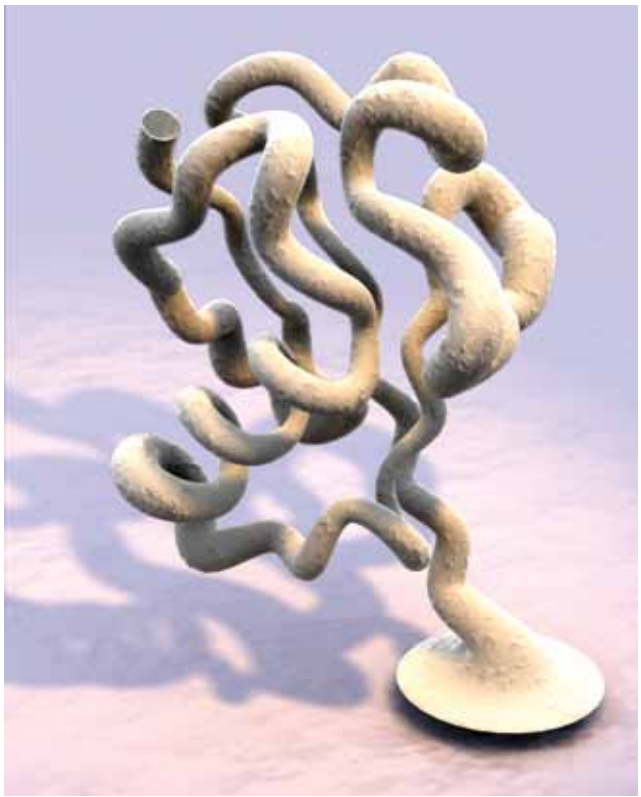
This chance survival of the less-than-fittest gives rise to random drift, whereby a population accumulates random, non-adaptive gene mutations. These mutations translate into 'wrong' amino acids in the peptide chain of the corresponding protein. In general, say Fernández and Lynch, that makes the protein's structure less stable.

In particular, mutations are likely to disrupt the way in which proteins shield hydrogen-bonded parts of their backbone from the surrounding water molecules. These hydrogen bonds can be crucial in pinning the protein's folded structure in place. But water molecules can intrude and compete for the hydrogen bonds, like lovers undermining a marriage.

To prevent this, most backbone hydrogen bonds in proteins are 'wrapped' in hydrophobic groups, which repel water molecules and effectively 'dry' the hydrogen-bonded region. Fernández and his coworkers have previously found that positions on protein surfaces where they interact with other proteins are often poorly wrapped. They call such regions 'dehydrons'.[2] Many proteins possess dehydron units – human myoglobin has 16, for instance, and human ubiquitin has 12.

United we stand

The poor wrapping of hydrogen bonds in dehydrons allows water to interfere. But if two such regions come into close contact, the water nearby is squeezed out, and they are dry once more. Protein–protein associations thus commonly shield dehydrons. Fernández and Lynch now suggest that such sticky regions are likely to be accidental, arising from the mutations of random drift.



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Human ubiquitin has 12 exposed 'dehydrons' and is involved in many protein–protein interactions

The two researchers compared water-soluble proteins in humans with those sharing a common molecular ancestor in very different species, such as bacteria; these proteins are known as orthologues. The basic folded shapes of orthologues are similar, even though their amino acid sequences can differ substantially. But when they examined the structures closely, the duo noticed that the structure in proteins from species with smaller populations seemed looser – less well packed, with more dehydrons on their surface.

What's more, this disruption and loosening of the proteins increased as the number of protein–protein interactions in the species increased. This suggests that the interactions didn't evolve by natural selection – they 'just happened', because random drift created dehydrons that were then attracted to other dehydrons, or to other hydrophobic regions of a protein that improve the wrapping. In effect, it is drift that made the proteins sticky.

Runaway bureaucracy

The upshot is, says Fernández, that 'complexity is not really selected for but arises because of selection inefficiency'. He points out that this challenges 'our dogmatic way of attributing any complex trait to the workings of natural selection'. Rather, he says, 'natural selection and evolution are not the same thing. Selection is by no means the only force contributing to evolution'.

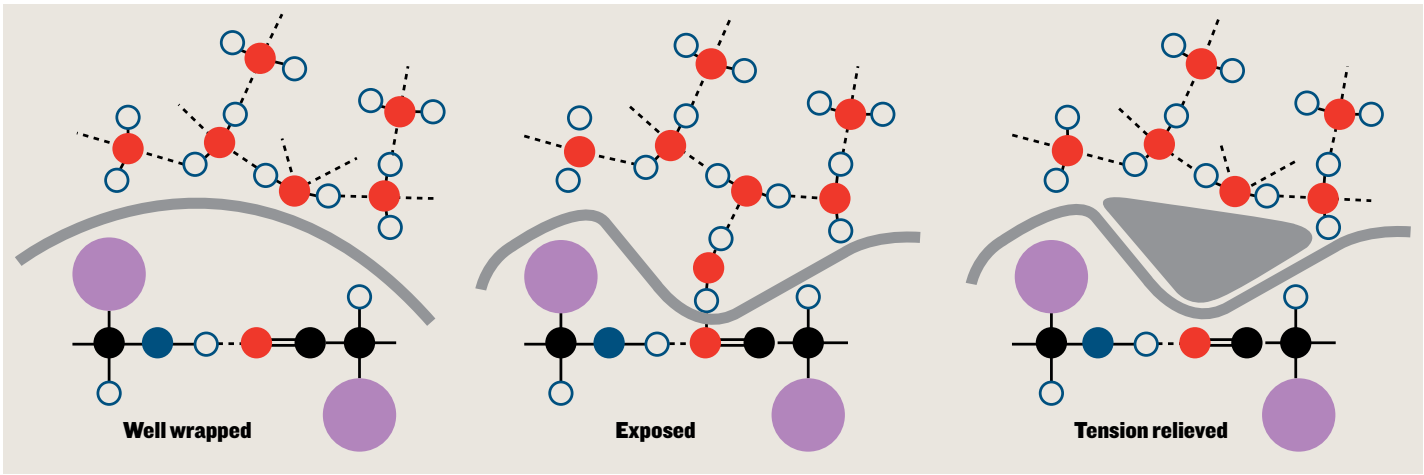
'Natural selection and evolution are not the same thing. Selection is by no means the only force contributing to evolution'

Lynch has previously argued that molecular complexity happens via a kind of ratcheting effect, in which non-adaptive complications of the protein and gene machinery of cells become very hard for evolution to undo, unless there is a strong selective pressure for it.[3] Such 'neutrally adaptive fixation' – much more prevalent in small populations due to drift – might account for such apparently over-complex features as transposons (genetic elements that hop around the genome), introns (bits of DNA that need to be edited out before the corresponding RNA is translated into a protein) and other seemingly wasteful uses of DNA in eukaryotes. Commenting on this notion last year, Michael Gray of Dalhousie University in Halifax, Canada, and his coworkers explained that 'although complexity in biology is generally regarded as evidence of "fine tuning" or "sophistication", large biological conglomerates might be better interpreted as the consequences of runaway bureaucracy'.[4] Now Gray feels that Fernández and Lynch have offered a general mechanism for how this might work. 'I would assert that non-adaptive and adaptive mechanisms of evolution are complementary,' he says, 'and that both are essential.'

While Lane feels that Gray, Fernández and Lynch are on to something, he is not persuaded that the response to random drift is the major cause of complexity. 'There is plenty of scope for selection to do its stuff even in very small populations,' he says. He points out, for example, that sexual recombination of genomes counteracts the negative effects of drift in small populations, although 'maybe sex is not enough, and you need protein interactions too'. He also argues that expressing lots of proteins, and therefore more interactions between them, may be made easier in eukaryotes because their mitochondria reduce the energetic cost.[5]

Danger signs

In fact, Fernández and Lynch agree that selection operates in their model, but only after drift has created protein–protein interactions and interactome complexity. For example, once a 'beneficial' association has occurred, preventing



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water from loosening the proteins, further mutations might take place that encourage and stabilise the pairing. In this way, evolution can act to further preserve the function and interaction of genes whose proteins have initially come together for a non-adaptive reason.

By analysing proteins from species that have only recently diverged, Fernández and Lynch confirmed that this sort of secondary selection seems to take place. 'Random drift creates the evolutionary niche or opportunity for natural selection,' explains Fernández. In effect, nature might be considered to be making the best of a bad deal: rather than trying to fight the problem of dehydrons created by drift, it makes use of their tendency to create protein interactions. That might sound like a good idea, but in fact it could be dangerously short-sighted.

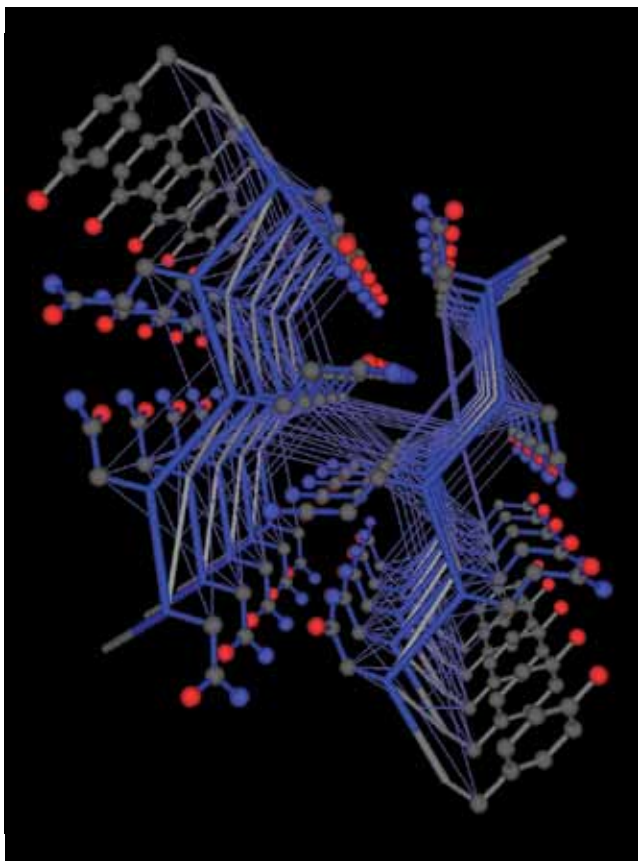
The problem is that natural selection can only look one step ahead, and can't plan for the distant future. This is no doubt why it goes down blind alleys and leads to extinctions. But perhaps it is taking us down one now. By masking the problem of dehydrons, the complexification of the genome allows such deleterious mutations to keep accumulating. Eventually, there may be too many of them to shield through protein associations, and the proteins themselves might start to unwind.

There are already hints that this can happen. Dehydrons seem to be a common feature of proteins apt to form amyloid aggregates, which are associated with neurodegenerative diseases such as Alzheimer's, Parkinson's and Huntington's diseases. Such proteins appear to undergo a conformational change from a soluble, globular form to insoluble aggregates. Prion diseases such as scrapie and Creutzfeld–Jakob disease may be caused by such a conformational change in the prion protein PrP. Fernández has previously proposed that destabilisation of the globular fold is related to the tendency of dehydron units to promote aggregation.[6]

Could it be that these diseases are the result of mutations created by random drift, and tolerated by natural selection via the complexity strategy? Fernández thinks so. 'This extreme case of an aberrantly needy protein [the prion] illustrates the level of gambling and the risks that nature is exposing us to by promoting the partial degradation of the protein structure as an evolutionary strategy to achieve complexity,' he says. 'It gives us clues as to where nature's gambit may lead our species to. I believe prions are indicators of a gambit gone too far.' If random mutations continue to do their dastardly work, he says, 'we as a species may end up facing more and more fitness catastrophes of the type that prions represent'.

When the 'wrapping' of hydrogen bonds is disrupted by a mutation, another protein can shield against attacking water

Protein aggregates like the amyloid plaques of Alzheimer's and prion diseases could be a sign of a gambit gone too far



ARIEL FERNÁNDEZ

But it's not all gloom. In the short term, we can benefit by drawing lessons for protein design, for example in making synthetic proteins that can self-assemble in particular ways, or that will bind to natural target proteins. Fernández says his results show that 'protein architectures that are likely to promote interactivity or associations are not rigid, but contain floppy regions belonging to a twilight zone between order and disorder'. We should look to human proteins, not bacteria, for examples of those. 'It doesn't seem far-fetched to envision biotechnology based on a controlled enhancement of selection inefficiency,' he says. 'Whether this is going to be the way of the future will depend on our ability to harness nature's lesson.'

Philip Ball is a science writer based in London, UK

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