

## STRUCTURAL BIOLOGY

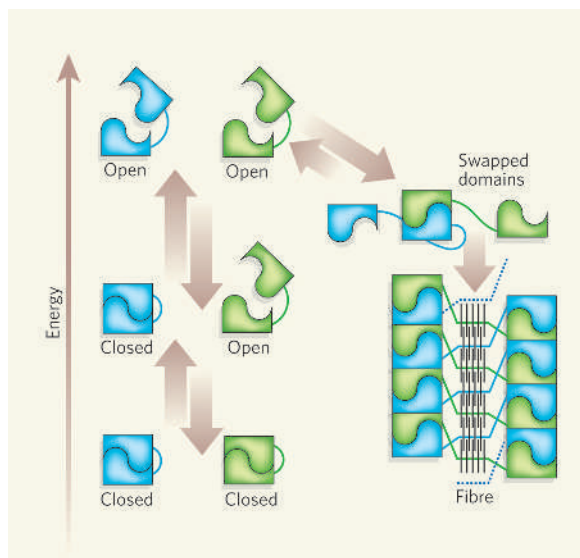
# Fibres hinge on swapped domains

Andrew D. Miranker

**When proteins assemble themselves into fibres, there can be grave pathological consequences. Designing an otherwise soluble protein to make fibres provides a general mechanism for the construction process.**

DNA encodes RNA, which encodes protein — this ‘central dogma’, coined by Francis Crick in 1958, underpins modern molecular biology<sup>1</sup>. Implicit in this formula is the tenet that a linear chain of amino acids contains the complete instructions to form a three-dimensional protein structure. Despite an astronomical number of possible configurations, a functional protein independently adopts the structure with the lowest free energy<sup>2</sup>. These axioms are so universally observed that their violation holds particular fascination. A functional example of this is  $\alpha$ -lytic protease, whose unfolded state is more stable than its native conformation<sup>3</sup>. A pathological example of where the rules appear to be broken is when proteins aggregate into a form that is more stable than their functional precursors — a process found in a host of diseases, such as Alzheimer’s, Huntington’s and prion diseases<sup>4</sup>. In this issue, Sambashivan *et al.* (page 266)<sup>5</sup> reconcile this apparent contradiction between dogma and observation.

The fact that normally soluble proteins are capable of aggregating comes as no surprise to anyone who has worked with them — finding a solid white mass at the bottom of your test tube is a well-known frustration of the field. Careful analysis of this phenomenon, however, reveals that in many cases the aggregates are structured, linear assemblies typically called amyloid fibres. Regardless of the starting conformation of the precursor, amyloid fibres are always formed about a central core whose polypeptide chain is organized from strands that stack at right angles to the long axis of the fibre. The initial formation of such structures is a rare event. Once present, however, the fibres form templates that catalyse their own formation. The resultant structures are exceptionally resistant to degradation and disassembly by chemical or proteolytic means. But hang on — a protein structure that requires a protein template? Alternative structures with greater stability than the



**Figure 1 | Structures and energies associated with assembly of domain-swapped proteins.** Some proteins adopt a structure characterized by two well-defined domains tethered together by a loop. If the loop can serve as a hinge, the protein may open, transiently adopting a high-energy state. Very rarely, two such open states encounter one another. This can result in the formation of an open swapped dimer where the interface of the two domains is regenerated, but by two separate polypeptides. Sambashivan *et al.*<sup>5</sup> engineered the hinge to include a short sequence that interacts with itself. The result is synergy between runaway domain swapping and the formation of an amyloid fibre backbone. (Adapted from Fig. 1c of ref. 5.)

native state? That goes against the dogma!

The proteins that behave like this are typically divided into two categories: those that assemble from globular precursors, such as  $\beta_2$ -microglobulin which deposits as amyloid fibres in patients treated with dialysis; and those that assemble from peptide precursors, such as amyloid- $\beta$  peptide, a peptide of 40–42 amino acids that forms fibres in the plaques of Alzheimer’s patients. In cases of the former, amyloid formation appears to result from the assembly of states that have both amyloid- and native-like features<sup>6</sup>. This suggests a role for native structure in amyloid assembly. In cases of the latter, the intrinsic disorder of the short flexible chain yields to the formation of a well-defined structure upon aggregation.

Sambashivan *et al.*<sup>5</sup> reconcile these two

categories of amyloid formation with a mechanism that does not violate the protein-folding dogma. The proposal is based on three-dimensional domain swapping<sup>7</sup>. In this mechanism, a single polypeptide chain can be thought of as having two independently folded sub-domains bound together through complementary surfaces (Fig. 1). The two domains are separated by a portion of the protein that acts as a hinge. Occasionally, the hinge will open and close, transiently exposing the interface. The interface can also be reconstituted through collision with a second protein that, fortuitously, is also in the open state. This is a rare event as it requires the collision of two high-energy, and therefore poorly populated, states. If the dimer forms in such a way that only one of the two open interfaces is closed, a state arises with sticky ends. This is the nucleating structure, because it always presents an open overhang to bind to other polypeptides. Furthermore, this open domain-swap is a structure-specific template of its own extension.

The problem with domain swapping as an explanation for fibre formation is that it is energetically unfavourable. A collection of proteins will have more entropy if they are free to move and tumble independently. Swapped assemblies lose this entropy in exchange for an interface that was already present in the starting structure. Therefore, there must be something about a swap that makes it more favourable to be joined up than to be free and single. The key is in the hinge. One possibility is that the hinge itself can form interactions with other hinges. Sambashivan *et al.* investigated this using hinges that can interact with each other, and introduced them into a protein that is capable of swapping domains.

Because of their large size and variable length, amyloid fibres confound traditional methods used to determine protein structure. Sambashivan *et al.*<sup>5</sup> use an ingenious approach to show that the protein in the fibre has a native structure. The design capitalizes on the

intrinsic ability of the enzyme RNase to form domain-swapped dimers. The active site of this enzyme includes two amino acids that are essential for catalysis and that reside on opposing domains. Mutating these amino acids separately generated two pools of inactive mutants. Upon domain swapping, these formed a pool of dimers, one in four of which is active — representing the probability of assembly of complementary mutants. The authors then engineered the hinge region to include the insertion of ten glutamine residues. Alone, this is a weakly amyloidogenic sequence; in the proteins that are mutated in polyglutamine-expansion diseases, such as Huntington's disease, stretches of more than 40 glutamines are required for significant aggregation<sup>8</sup>. Remarkably, a mixed assembly that includes the active-site and hinge-insertion mutations results in the formation of amyloid fibres with an enzymatic activity that is indicative of domain-swapped assembly.

Enzymatic activity is the unquestioned hallmark of a defined protein structure. In the founding days of protein crystallography, serious scepticism existed concerning the relevance of crystal structures to biological

function. Then, as now, RNase played a pivotal role in a seminal study<sup>9</sup> that established that proteins in crystals can be enzymatically active. There is considerably more mystery to be solved in the formation of amyloid fibres — for example, the structural nature of transient intermediates and the basis of their cytotoxicity. The current study<sup>5</sup> shows us that a molecular basis for these phenomena, with creative perseverance, is ultimately obtainable. ■

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1. Crick, F. H. C. *Symp. Soc. Exp. Biol.* **12**, 138–163 (1958).
2. Haber, E. & Anfinsen, C. B. *J. Biol. Chem.* **237**, 1839–1844 (1962).
3. Sohl, J. L. *et al. Nature* **395**, 817–819 (1998).
4. Dobson, C. M. *Nature* **426**, 884–890 (2003).
5. Sambashivan, S., Liu, Y., Sawaya, M. R., Gingery, M. & Eisenberg, D. *Nature* **437**, 266–269 (2005).
6. Eakin, C. M., Attenello, F. J., Morgan, C. J. & Miranker, A. D. *Biochemistry* **43**, 7808–7815 (2004).
7. Liu, Y. & Eisenberg, D. *Protein Sci.* **11**, 1285–1299 (2002).
8. Scherzinger, E. *et al. Proc. Natl Acad. Sci. USA* **96**, 4604–4609 (1999).
9. Doscher, M. S. & Richards, F. M. *J. Biol. Chem.* **238**, 2399–2406 (1963).

The force required to move a large, slow-moving plunger is proportional to its speed; all the mechanical work performed is lost, irretrievably, to the environment as heat. At a given speed, the work required to move the plunger between two well-defined states is always the same: viewed microscopically, the resistance to the motion is just the statistically averaged effect of many collisions between the plunger and the fast-moving molecules of the fluid.

If the plunger is very small, however, this average is performed over far fewer collisions, and the random, brownian movements of the plunger become significant. When an excess of molecular collisions occurs in the direction in which the plunger is moving, the work done by the operator is reduced. This work can on rare occasions be reduced to zero, or even become negative — meaning that the system performs work on the operator. The mechanical work needed to move a small plunger is no longer a precise, deterministic variable; rather, it is described by a statistical distribution.

The CFT stipulates that the probability of exerting a given amount of mechanical work on the plunger in the conventional, forward process, divided by the probability of getting the same amount of work back from the reverse process, depends exponentially on the amount of work lost to the environment. This implies that the likelihood of dissipating virtually no work is the same in both the forward and reverse directions. Because the amount of work dissipated increases as a system gets larger, in a sufficiently small system the likelihood that 'dissipation-free' events will occur becomes large enough for them to be observed directly.

Collin *et al.*<sup>1</sup> studied the mechanical unfolding and refolding of individual RNA molecules, applying and measuring forces using optical tweezers — a focused laser beam that acts as an ultra-sensitive spring to trap and hold small particles. The two ends of an RNA molecule, folded into junctions and hairpin shapes, were each linked to a small bead, one held by a micropipette and the other by the optical tweezers. In each experimental cycle,

## BIOLOGICAL PHYSICS

# Rare returns on lost effort

Wesley P. Wong and Evan Evans

**How does the size of a system affect its thermodynamic irreversibility? A deft experiment that observes the unfolding and refolding of a single molecule of RNA provides insights into the question at a small scale.**

When vigorously stirred, a cup of coffee will heat up; a cup of hot coffee, however, will never stir a spoon (Fig. 1). This type of irreversibility is a cornerstone of macroscopic thermodynamics. But does it still hold when the spoon is extremely small? Newly discovered relationships between heat, work and energy have revealed unexpected features of small systems when they are driven far from equilibrium — relationships that are becoming increasingly relevant as experiments probe ever-tinier systems, including the nanoscale machinery of living cells. On page 231 of this issue, Collin *et al.*<sup>1</sup> describe a single-molecule experiment that not only validates one prominent recent postulate, known as the Crooks fluctuation theorem, but also provides a new method for quantifying the difference in equilibrium free energy — the useful work expected to be extracted from a system — between two biomolecular states.

The Crooks fluctuation theorem<sup>2</sup> (CFT) describes the exchange of energy between a system and its environment in forward and reverse processes. It generalizes the so-called Jarzynski equality<sup>3</sup>, which relates equilibrium

free energy to the work done in multiple, non-equilibrium measurements. The application of the CFT to a single-molecule experiment can be understood by considering an idealized 'dashpot' — a plunger that can move through a viscous fluid held at a constant temperature.



**Figure 1 | Stirring stuff.** A paradigm of thermodynamic irreversibility: the world's largest cappuccino.

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