pyridine-2-carboxaldehyde (blue, Fig. 1b), and zinc triflate resulted in the *in situ* formation of complex **1**.

This one-pot reaction solved both the thermodynamic challenge — an equilibrium constant of 10^8–10^9 M^{-2} clearly favoured the complexed alcohol — and significantly improved the kinetics as this assembly is fully formed after stirring overnight in acetonitrile at room temperature. The dynamic nature of the assembly was subsequently demonstrated by showing that both the aldehyde and alcohol components could be readily exchanged. Mechanistic and mass spectrometric studies suggested that the iminium ion framed in Fig. 1b acts as the key intermediate in both aldehyde and alcohol scrambling, and found no evidence of the alternative (hemi)acetal intermediates that would be formed by the initial direct reaction between the alcohol and the aldehyde.

Anslyn and co-workers also demonstrate the first practical application of this new example of DCC. With their long-standing interest in the supramolecular analytical chemistry of chiral species[4], the team was interested in the challenge of developing an optical method for identifying the chirality of simple mono-alcohols. If isopropanol is replaced with an enantiomerically pure alcohol, a pair of diastereomeric complexes is formed that can be identified with ^{1}H NMR spectroscopy. Diastereomeric ratios of 1.3–2 were observed, depending on the nature of the alcohol. Next, the influence of the chirality of the ligand over the twist at the Zn(II) centre was used to identify chiralities of the optically active alcohols using circular dichroism (CD). It was found that, among the three alcohols that were examined, all (*S*)-enantiomers induced positive Cotton effects at 269 nm, whereas their (*R*)-counterparts resulted in negative Cotton effects. This method was then successfully used to quantify the enantiomeric excess (e.e.) of different mixtures of (*S*) and (*R*) alcohols. An excellent correlation ($R^2 = 0.99$) was established between the intensity of the CD signal and the e.e. of the mixtures, and the absolute error of this method was estimated at 2.3%. This purely optical method is sufficiently accurate to be used to rapidly screen e.e. values of mixtures of products obtained in asymmetric catalytic reactions.

This work offers ample inspiration for future studies in DCC. Of immediate interest would be to establish whether sterically more demanding tertiary alcohols and ketones could engage in similar chemistry. Moreover, the redox relationship between ketones and secondary alcohols could perhaps be used to combine reversible alcohol coordination with its irreversible oxidation into complex sequences that have recently been shown to induce self-sorting behaviour[5]. Incorporation of alcohols into dynamic organic assemblies will enable their use for the coordination of oxophilic metals and could open up new avenues analogous to those realized by the well-established dynamic imine–metal chemistry[6,7]. There could also be benefits in using less strongly coordinating metals to intentionally destabilize the complex with the hemiaminal ether. Given the broad use of iminium activation in organocatalysis, could this method be used to screen for and identify new catalytic reactions of iminiums?

Finally, this study is the latest demonstration in support of the notion that aldehydes are quickly evolving into the 'crossroads species' within the domain of DCC: imine, acetal, hydrazone and now hemiaminal ether chemistry all use aldehydes as partners in the construction of dynamic structures. This convergence could be explored in the preparation of complex mixtures that express one class of aldehyde condensates under a given set of conditions and a completely different one if conditions (for example, the chelating metal) are changed. Such ability to *de facto* regulate[5,8] dynamic combinatorial libraries will further distinguish this methodology from relatively inflexible kinetically controlled reactions, bringing DCC closer to the sophisticated and controlled operation of metabolic pathways. ❐

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PROTEIN THERMODYNAMICS

Are native proteins metastable?

Thermodynamic measurements show that the most stable structural form of a number of proteins under cellular conditions is fibrillar, implying that their functional states may only be metastable.

D. Thirumalai and G. Reddy

It is well accepted that to function proteins must fold into specific three-dimensional structures. Even under physiological conditions the functionally competent states of proteins are suspected to be the most stable thermodynamically, corresponding to the global minimum in the free-energy landscape. On the other hand, numerous *in vitro* experiments have shown that as the concentration of the protein increases beyond a critical value, proteins aggregate to form amyloid fibrils, adopting conformations that are rich in β-sheet content regardless of the sequences or the structures of the isolated monomers. Moreover, proteins that escape degradation could accumulate in the cellular or extra cellular regions thus becoming susceptible to fibril formation. Thus, at high concentration of proteins, the structure of the protein in the fibril is thermodynamically more stable than all other states. Because the aggregated forms of several proteins and peptides are linked to many pathological disorders, such as Alzheimer's and Parkinson's diseases, there has been intense effort to understand how proteins have evolved to avoid aggregation during their normal lifespans *in vivo*.

Our understanding of protein aggregation raises the question: are cellular concentrations of proteins sufficiently high such that they are always poised to form amyloid fibrils? A thought-provoking work from Christopher Dobson and co-workers[1], published in the *Journal of*

American Chemical Society, answers this question by measuring the solubility limit of monomers above which fibrillization is facile. Remarkably, they found that the cellular concentrations of free, functional proteins with sequence length $L < L^* \approx 150$ are high enough that they ought to form ordered amyloid fibrils at equilibrium. Therefore, they surmise that the native states of these proteins must be metastable *in vivo* with respect to the fibril structures[1]. An immediate consequence of their finding is that large kinetic barriers between the folded functional states to aggregation-competent structures must exist (Fig. 1), which prevent transitions to the aggregation-prone structures during the lifetimes of proteins. Thus, under cellular conditions folding, and presumably function, could be under kinetic control. Because the free energy of formation of fibrils is less favourable as L increases, Dobson and co-workers[1], conclude that there must be evolutionary pressure to generate long protein molecules at least in complex organisms.

The conclusions reached are based on the study of a number of proteins for which they extract the solubility limit M_s^{max} of free monomers that are in equilibrium with a fibril. This allows them to determine the free-energy gain (ΔG^0_{fib}) per fibril monomer with respect to the free soluble monomers *in vitro*, and gives a handle on the limit of stability of free monomers in isolation.

Wetzel and co-workers[2] showed previously that by assuming that only soluble monomers and fibrils are present at steady state, knowledge of M_s^{max} can be used to obtain the associated equilibrium constant K. Dobson and co-workers used essentially the same procedure introduced by Wetzel[2], who showed that when steady state is reached in the growth of fibrils M_s^{max} does not change for a wide range of the total protein concentrations. If the fibril is insoluble then $M_s^{max} = 1/K$ and $\Delta G^0_{fib} = -RT\ln K$.

To obtain the free energies, Dobson and co-workers used the dependency of K on the concentration of denaturants, D. Consequently, the elongation free-energy in the fibrillar state with respect to the soluble monomer state is $\Delta G_{fib} = m[D] + \Delta G^0_{fib}$, where m is a measure of the cooperativity of the monomer to fibril conversion. Dobson and colleagues[1] used the Oosawa linear polymerization growth model[3] to connect the measurable M_s to the $[D]$-dependent equilibrium constant. By measuring M_s as a function of $[D]$ they estimated ΔG_{fib} from which ΔG^0_{fib} can be obtained assuming that linear free-energy relation holds. The Wetzel procedure[2] and the one used by Dobson and colleagues[1] are equivalent provided K is large.

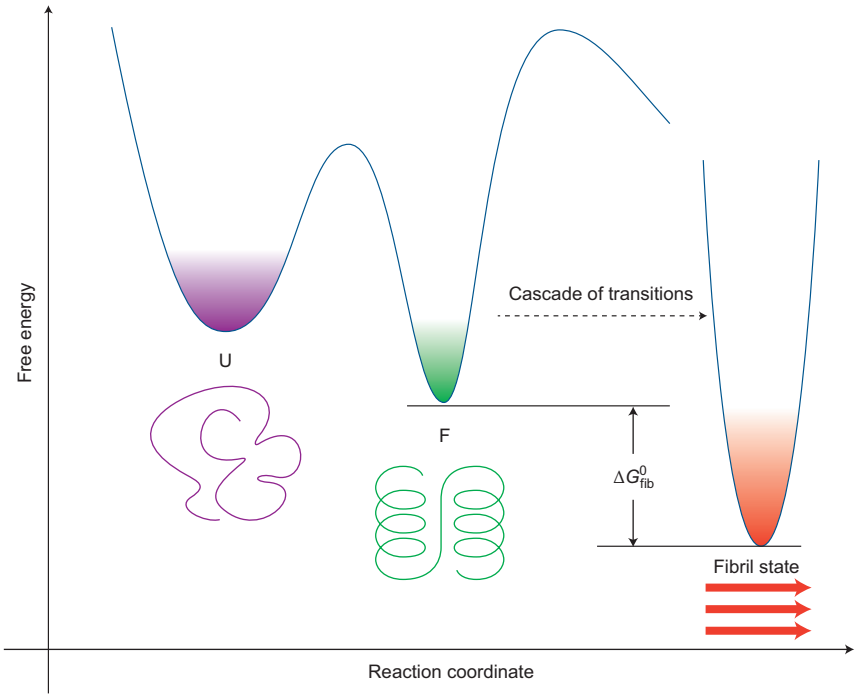

Figure 1 | Schematic energy landscape for folding and aggregation. The free-energy difference between the soluble monomers and the fibril, ΔG^0_{fib}, is determined by the limit of solubility of the monomers. Soluble monomers can be either unfolded (U) or folded (F). If $\Delta G^0_{fib} < 0$ then the folded state is metastable with respect to the fibril state.

The critical concentration of the protein, below which the fibril state is unstable, is obtained using $M_s^{max} = \exp(\Delta G^0_{fib}/RT)$. Surprisingly, Dobson and colleagues found[1] that when they compared their measured values of M_s^{max} with *in vivo* concentrations taken from the literature, the physiological concentrations exceeded M_s^{max} for a number of proteins. For example, for lysozyme, the cellular concentration is nearly 50 times greater than M_s^{max}, which implies that fibril structure must be the lowest free-energy structure. Thus it is surmised that the functionally competent states of many proteins must be metastable with respect to the fibril. The analysis also suggests that due to topological frustration longer proteins are unlikely to form amyloid fibrils. Interestingly, several proteins that are associated with diseases are smaller than L^*.

The inference that the native states of proteins could be metastable is not without precedence[4,5]. Moreover, it has also been suggested that the cellular form of the predominantly helical mammalian prions are metastable[6] with respect to an assembly-competent structure that is rich in β-sheet. However, the study by Dobson and co-workers[1] is unique because their conclusions focus on the importance of kinetics in cellular processes. What matters for function is that aggregation should not occur during the life cycle of a typical protein. Thus if the timescale for fibril formation, which is a multistep process, (Fig. 1), is long compared with the lifespan of a protein then the reduced stability of the folded monomer relative to the fibril is irrelevant. Moreover, multiple regulatory mechanisms are surely involved in imparting kinetic stability to folded proteins in cells. These quality-control mechanisms are known to play a key role in proteostasis[7] so that aberrant aggregation reactions are minimized. Additional studies are needed to ascertain whether factors known to be relevant in cells, such as macromolecular crowding and osmolytes, stabilize the folded state to such an extent that the fibril state is not the most stable (Fig. 1) even at elevated protein concentrations. ❐

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