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Proteins: molecules defined by their trade-offs

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Proteins are subject to various conflicting forces that trade-off against each other. For example, during folding, the protein achieves lower enthalpy at the cost of lower entropy. Similarly, the trade-off for increased stability may be decreased flexibility, which may abolish allosteric pathways. Accordingly, stability trades-off against function, which may also trade-off against folding kinetics and mechanism. Furthermore, attaining increased stability may reduce a protein's ability to adopt novel functions. Understanding the biophysics and function of proteins requires quantification of the driving forces involved in each of the trade-offs. Indeed, quantification of the linkages in the network of trade-offs is essential to obtaining a more complete understanding of protein structure and function.

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Introduction

Trade-offs have been observed in many areas of life, including in decision-making problems [1,2], the evolution of organisms [3], and the microscopic properties of biomolecules, such as proteins and DNA. Trade-offs occur when the attainment of two desired outcomes stand in contradiction to each other. For example, the dilemma of whether to wear gloves or mittens on a cold winter day reflects a trade-off between function and warmth. Mittens, in which all fingers are in the same compartment, keep the hands warmer, but at the expense of functionality, in that it is difficult to manipulate objects while wearing mittens. By contrast, gloves, in which each finger is in a separate compartment, provide better functionality but less warmth. In the gloves–mittens example, the trade-off is simple because it involves only two desired outcomes. When examining trade-offs in proteins, the list of desired outcomes is much longer, and therefore trade-offs in proteins form a complex network. Some of the trade-offs are intrinsic to any protein while others may depend on the protein sequence and structure.

In this opinion, we classify the desired protein outcomes into three categories: (1) biophysical properties; (2) functional properties; and (3) evolutionary properties. Trade-offs are found within these categories but mostly between them, indicating a potential tag-of-war between proteins' biophysics, function and evolution (Figure 1). In the following sections, we will discuss some of the common trade-offs within and between these three categories (Figure 2), and will conclude with strategies that nature and protein engineers have developed to circumvent some of the trade-offs inherent in protein evolution and design.

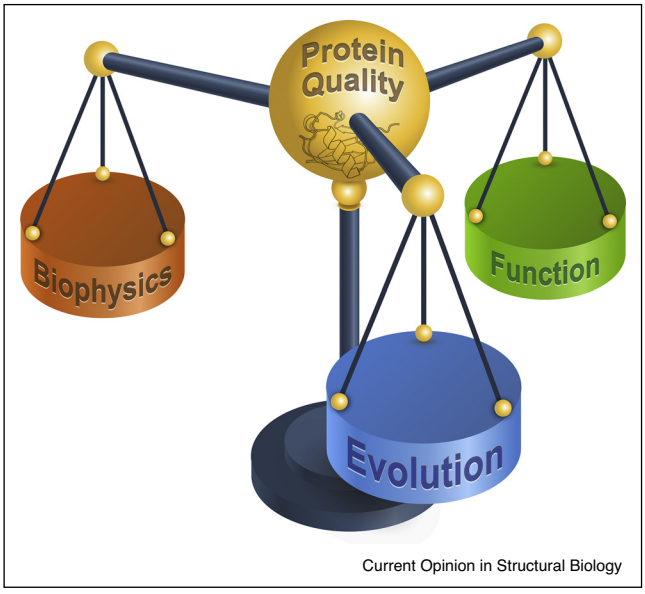
Entropy–enthalpy trade-off

Since most proteins usually need to adopt a specific structure to function, they must also be thermodynamically stable and kinetically foldable. For a protein to be thermodynamically stable, the free energy of its native state (G_N) must be lower than the free energy of its unstructured ensemble (G_U), such that $G_N - G_U = \Delta G < 0$. The low free energy of the native state can be achieved via low enthalpy or high entropy. However, enthalpy and entropy often trade-off in what is known as 'entropy-enthalpy compensation' [4] (Figure 3A).

The physical origin of entropy–enthalpy compensation in protein folding may be the rearrangement of water molecules in the protein's proximity [5]. That is, a perturbation that leads to the formation of more water-mediated hydrogen bonds in the protein will lead to a decrease in enthalpy but, at the same time, the new constraints that the water molecules impose on the protein also decrease the entropy of the system. Alternatively, entropy–enthalpy compensation could originate from the properties of the protein itself [4]. For instance, the formation of non-bonded interactions in the native state can lead to a decrease in the plasticity of the protein and hence to an undesired decrease in entropy.

Intuitively, the thermodynamic stability of proteins can be altered by modifying the enthalpy of the folded state (e.g., by point mutations) or the entropy of the unfolded state (e.g., by cyclization or disulfide bond formation). Several studies have demonstrated mechanisms for trade-offs between entropy and enthalpy that are less common. For example, loop truncation is a common protein modification, and it is suggested that loops have an important role in protein stability [6] and function [7]. Although, intuitively, loop truncation can result in higher stability by reducing the entropy of the unfolded ensemble, loop truncation can also

Figure 1



Proteins properties are an outcome of a tag-of-war between biophysical, functional, and evolutionary forces. Some of these forces might be in conflict and define trade-offs.

lead to an increase in the entropy of the folded state of the protein due to deletion of interactions loop residues have with other structural elements in the protein. This can lead to thermodynamic stabilization of the protein by decreasing the entropic loss of folding [8–10].

Multi-domain proteins are common in all kingdoms of life [11]. In some cases, multiple domains are encoded in the sequence of the proteins, but in other cases the additional domains are the result of post-translational modifications (PTMs), such as glycosylation [12], ubiquitination [13], or biotechnological applications (labeling with GFP). The effect of multiple domains on the thermodynamic stability of proteins is not always simply additive, and is system-dependent [14•,15]. In some cases, tethering a conjugate to a protein can lead to thermodynamic destabilization of the protein [16,17]. It was shown that destabilization of conjugated proteins can be caused by an increase in the entropy of the unfolded state. Moreover, tethering leads to elimination of residual contacts in the unfolded ensemble (increase in enthalpy) and to an increase in the configurational entropy of the protein [18•].

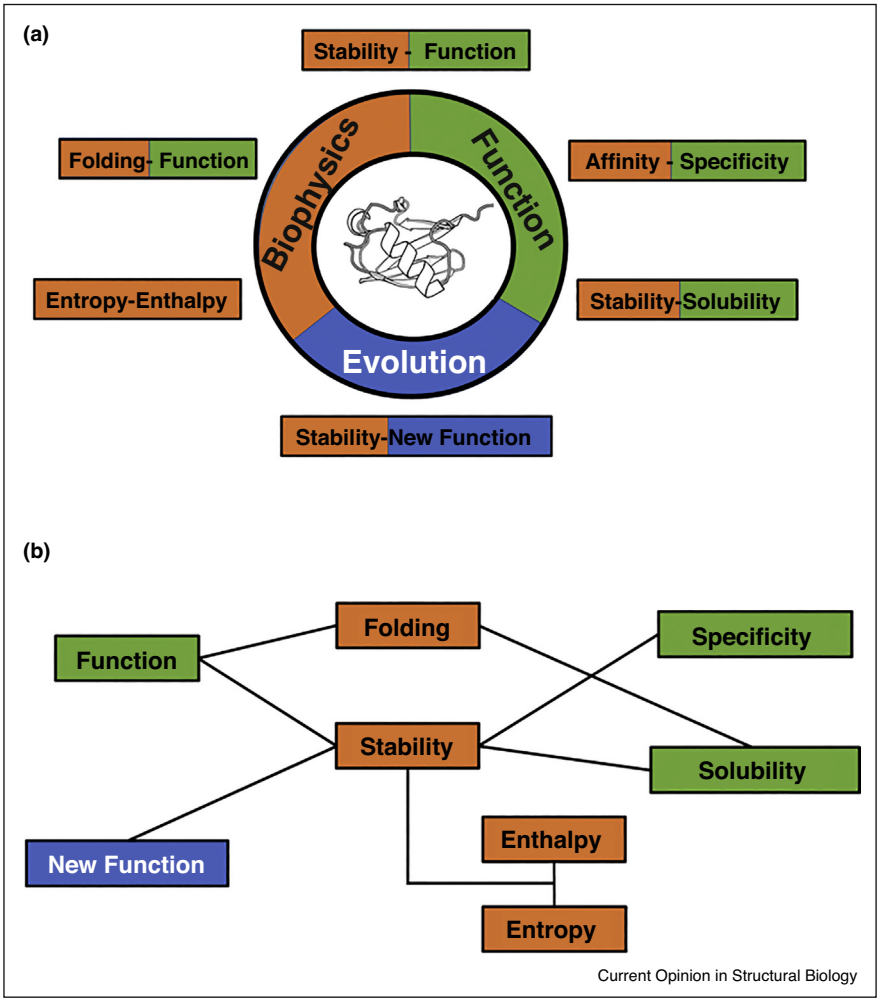
Another surprising example of the trade-off between entropy and enthalpy is the effect of mutations on protein thermodynamic stability. Replacing a wild-type protein residue with alanine can be regarded as the elimination of a contact between residues i and j. Considering the enthalpy of the native state, contacts with different sequence separation (i–j) are expected to have similar effects on protein stability. Hence, mutating residues with different sequence separations is expected to produce similar effects on protein thermodynamic stability. Surprisingly, there is evidence that deletion of contacts with large sequence separation (long-range contacts) leads to greater thermodynamic destabilization than the deletion of contacts that are near in sequence. Using computational, theoretical, and bioinformatic tools, it was found that this contact-length dependent destabilization originates from an increase in the entropy of the unfolded ensemble, which is not fully compensated for by an increase in the system's enthalpy following the elimination of residual contacts [19•]. Similar findings were reported for lattice models of proteins [20,21]. In addition, it was suggested that long-range contacts play an important role in increasing the protein folding rate [22–24].

Stability–function trade-off

Proteins are known to be only marginally stable, with typical ΔG values in the range of 5–15 kcal/mol [25]. This marginal stability, which originates from the trade-off between entropy and enthalpy mentioned above, is also linked to a trade-off between protein thermodynamic stability and other desired outcomes (Figure 3B). For example, in order for a protein to maintain its function in the native state, the free energy of the native state may be optimized for function rather than for thermodynamic stability. Indeed, early studies showed that the presence of stabilizing mutations in catalytic or binding sites decreased enzyme activity [26–28]. Similarly, it was found that stabilizing mutations in the binding sites of proteins significantly decreased binding affinity [29]. In another study, a variant of the fibronectin type III (FN3) protein, being a dimeric glycoprotein involved in several cellular processes, was constructed with picomolar affinity (six orders of magnitude more than wild type FN3), but the large increase in affinity came at the cost of a decrease in Tm of ~30°C [30]. Mutation at binding sites that enhance affinity of interaction with another biomolecule may affect function via changes in specificity. Although in some cases proteins have high affinity only to a specific ligand (i.e., high affinity and high specificity), other relationships between affinity and specificity exist (e.g., high affinity and low specificity or low affinity and low specificity) [31,32].

Increased stability can also come at the expense of activity when the stabilizing mutation is distant from the active/binding site. In these cases, a stabilizing mutation can lead to an overall decrease in the flexibility of the proteins and restriction of the conformational heterogeneity, which may affect the active site via allosteric pathways [33]. However, it was shown that stabilizing mutations occurring at locations distant from the active site do not necessarily lead to a decrease in function [34–36].

Figure 2

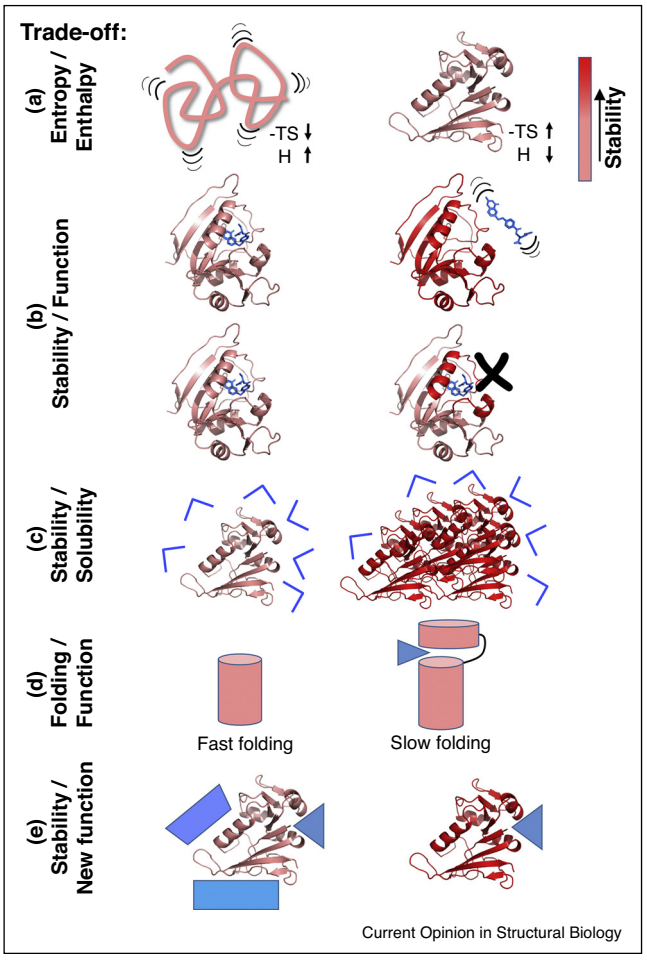


(a) Turning on protein trade-offs. Proteins are made of various trade-offs which can be classified as having consequence on protein's biophysical (orange), functional (green), or evolutionary (blue) properties. Some of these conflicting forces are inherent in proteins (e.g., trade-off between enthalpy and entropy); however, some are valid only in some proteins (e.g., trade-offs between folding and function or between stability and new function). We note that this list of trade-off does not aim to be complete, thus additional trade-offs may exist as well. **(b) Proteins are molecules subject to a network of trade-offs.** While balancing the conflicting forces in each protein trade-off is not trivial, one has to consider cross-talks between the trade-offs because they are linked. For example, the thermodynamic stability is in conflict with protein function, solubility, specificity and the ability to adopt a new function. This illustrates the complexity of the evolutionary forces on protein stability.

Stability–solubility trade-off

In order for proteins to remain functional in the cell, they need to remain soluble. When proteins are not sufficiently soluble, they may form insoluble aggregates. In the complex energy landscape of proteins, the native structure does not always reside in its global energy minimum (Figure 3C). Aggregates of various protein forms, which often have lower free energies than the protein's native structure, are related to more than 50 types of disease [37]. The disease-related proteins, some globular and some intrinsically disordered, form fibrillar aggregates with a cross-beta structure, known as amyloids. Under native conditions, proteins are usually blocked from transitioning into the aggregated state by a large free-energy barrier [38]. However, the protein energy landscape can be perturbed by the imposition of various forms of stress [39,40]. These perturbations can enable protein aggregation either by permitting the protein to transition to partially/completely unfolded states, which readily aggregate, or through the effects of even very local protein unfolding events [41]. In addition, several mutational studies of the aggregation propensity of globular proteins show that greater thermodynamic stability in proteins tends to reduce their aggregation [42,43], even in live cells [44,45]. Although some studies show that mutations that decrease net charge [46] or increase hydrophobicity and the propensity to form beta-sheets [47] increase aggregation propensity, this is not always the case [42,43].

Figure 3



Schematic illustrations of trade-offs in proteins. Entropy/enthalpy trade-off: the high entropy (i.e., low –TS) and high enthalpy in the unfolded state is exchanged with low entropy and low enthalpy in the folded state. **Stability/function trade-off**: Stabilizing the protein may result in lower affinity to ligands (e.g., the interaction of Dihydrofolate Reducatse with Dihydrofolate) due to a global change in flexibility and consequently in allostery. The function/stability trade-off is also highlighted by the observations that mutating catalytic residues (i.e., loss of enzymatic activity) may often increase stability. **Stability/solubility trade-off:** increasing stability may reduce protein solubility and result with aggregation. **Folding/function trade-off:** Some domains that are directly involved in function (e.g., binding a ligand, represented by the blue triangle) increase the free energy barrier for folding and result in slower folding. **Stability/New function trade-off:** increasing protein stability may eliminate the ability to acquire new functions (e.g., the evolvability to interact with various ligands). The stability of the protein is introduced by the pink-to-red colorbar.

It appears, therefore, that thermodynamic stability and solubility do not trade-off in a consistent manner. They are two sides of the same coin rather than two ends of the same rope. Nonetheless, when a meta-predictor that combined 11 different prediction algorithms was used to design stable proteins, the result was proteins that were more stable but less soluble. This trade-off occurred because stabilization was achieved via the addition of hydrophobic residues to the protein surface [48•]. The physical origin of stabilization in this case may arise from hydrophobic side chains that bury more surface area in the native state than in the unfolded ensemble. Similarly, it was found that, in the presence of the HSP90 chaperone, a viral protein (P1, from poliovirus) undergoes mutations that increase its stability, but also increase its aggregation propensity. The mutations were found to involve mostly hydrophobic residues [49].

Folding–function trade-off

One possible strategy by which proteins could minimize their propensity to aggregate is to fold rapidly down a smooth energy landscape. Proteins that fold rapidly have a lower chance of encountering other proteins in the cell before they fold, and hence a lower aggregation propensity [50]. However, in some cases, fast folding trades-off against function. For example, it was shown that shortening a loop in the pin1 WW domain speeds up protein folding by up to an order of magnitude in comparison with the wild-type, but eliminates the ability of the protein to bind ligands [51]. Similarly, it was suggested that functional constraints lead to the observed ruggedness of the energy landscape of Im7 [52], frataxin [53] and β-trefoil protein IL-1β [54,55•]•. Interestingly, the addition of a function to a functionless motif of IL-1β has a dual effect: when a function is added through the addition of new structural elements, folding is slower and more complex (Figure 3D). The origin of the high free-energy barrier for folding when a functional domain (β-bulge loop in the case of IL-1β) is introduced is linked to backtracking that is required to resolve unproductive folding events associated with this domain, namely slower folding kinetics [54,56]. However, when a function is added by using existing structural elements (e.g., for the protein Hisactophilin that has a β-trefoil structure but folds faster than IL-1β), the folding is less complex, occurs faster, but the folded protein is also less stable [55•].

Stability–new function trade-off

An important property of proteins is their ability to evolve to gain improved or new functions. In accordance with the observation that protein thermodynamic stability and function often trade-off, it was shown that mutations that provide new or improved functions also decrease stability [57,58] (Figure 3E), often because they lead to the exposure of polar or charged residues to a hydrophobic environment. However, these observations may not indicate that there is a general trade-off between stability and new functions, since most mutations are known to be destabilizing. A more precise description may be that mutations leading to new functions are more destabilizing than mutations that do not bequeath new functions (neutral mutations) [59,60]. One possible outcome of this observation is that neutral mutations can increase stability and hence avoid the loss of stability introduced when

mutation adds a new function. Another outcome may be that proteins that are more stable than their counterparts are more likely to evolve new functions since they can tolerate more mutations while retaining sufficient thermodynamic stability to function [61,62].

Circumventing protein trade-offs

In light of the complex network of trade-offs in proteins reviewed above, it is interesting to examine what strategies nature uses to navigate within the trade-off network (Figure 2) and to optimally balance between different desired outcomes. In the network of trade-offs, thermodynamic stability emerges as a central property that several other desired outcomes trade-off against. As a result, globular proteins exhibit a relatively poor thermodynamic stability. Breaking the delicate balance between the various desired protein properties can lead to undesired outcomes, such as misfolding, aggregation and degradation (which in some cases is not wanted).

One navigation strategy is to favor function over thermodynamic stability. This strategy is adopted by intrinsically disordered proteins (IDPs), which lack a thermodynamically stable 3D structure, but have an important functional role [63]. IDPs are abundant in the eukaryotic proteome, are known to serve as hubs in protein interaction networks, and play a central role in the regulation of signaling pathways.

While some of the trade-offs are intrinsic to proteins (e.g., entropy-enthalpy trade-off in folded proteins), others (e.g., stability-function or foldability-function trade-offs) may depend on the protein sequence and may originate from the constrained chemical-space introduced by the 20 amino-acids. PTMs can be viewed as a means to manipulate these trade-offs by expanding this chemical space. Indeed, different PTMs such as phosphorylation [64] glycosylation [65,66] and myristoylation [67] were shown to affect protein stability and folding, illustrating that the trade-offs can be delicate and are tunable.

Another strategy to navigate within the trade-off network is to use molecular chaperones in order to increase the probability of proteins to reach the thermodynamically stable, functional state [68]. Most chaperones bind disordered protein regions and use energy from ATP hydrolysis to perform their function, which differs between chaperone families. These functions include disaggregation, translocation across organelle membranes and assisting protein folding.

In addition to the paths that nature takes in the protein trade-off network, protein engineers and designers have embedded knowledge of trade-offs into their design efforts with the goal of circumventing trade-offs. Several studies have shown that it is possible to design a protein with improved thermodynamic stability and improved functionality (binding or catalysis) by following specific structural and/or evolutionary design rules [36,69–71]. One such rule is not to mutate residues located in the active/binding site of the protein when the aim is to increase activity/binding. Another is to avoid the introduction of hydrophobic residues onto the protein surface, as this is known to increase the aggregation propensity.

Conclusions

Various conflicting forces act on proteins and lead to several trade-offs, suggesting that proteins are frustrated objects [72]. While the trade-offs describe conflicts in macroscopic properties of proteins, conflicts can also exist at the microscopic level as was shown by the local frustration analysis and their connection to function [73]. Hence, trade-offs are an inherent property of proteins and are expected to be minimal. This is illustrated by the minimal frustration principle for protein folding that address the conflict between folding kinetics and stability [74,75]. The trade-offs are not only between the biophysical properties of the proteins but are also linked to their functional or evolutionary characteristics. A more complete understanding of protein complexity may require a 'Systems protein' approach that includes a quantification of their trade-offs. Obviously, optimizing protein trade-offs is more complex when considering the network of interaction between the trade-offs and the opposing forces that may act differently on different trade-offs. For example, while high stability trades-off with function as well as with the accumulation of new function it decreases the tendency to aggregate.

Knowledge about the various trade-offs in proteins has led to a deep understanding of the complex and delicate balance between various desired protein properties. However, most of the work discussed in this review dealt with small single domain proteins. Multi-domain proteins are more complex than single domain proteins and, as mentioned earlier, their biophysical and functional properties are not simply additive [14•]. We have shown that the trade-off between enthalpy and entropy for multi-domain proteins deviates from that of single-domain proteins [8,18•]. The trade-offs might be different if the domains in multi-domain proteins are inserted within another domain [76]. The magnitude of the trade-offs is expected to be affected by the protein topology (e.g., knots in the structure). Describing protein biophysics and function in terms of a network of trade-offs, especially in cases of large and multidomain proteins, may allow better understanding and trigger development of approaches to manipulate them.

Conflict of interest statement

Nothing declared.

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