Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Structural Biology

Physical and molecular bases of protein thermal stability and cold adaptation

Fabrizio Pucci[1,2] and Marianne Rooman[1,2]

The molecular bases of thermal and cold stability and adaptation, which allow proteins to remain folded and functional in the temperature ranges in which their host organisms live and grow, are still only partially elucidated. Indeed, both experimental and computational studies fail to yield a fully precise and global physical picture, essentially because all effects are context-dependent and thus quite intricate to unravel. We present a snapshot of the current state of knowledge of this highly complex and challenging issue, whose resolution would enable large-scale rational protein design.

Addresses
[1] Department of BioModeling, BioInformatics and BioProcesses, Université Libre de Bruxelles, CP 165/61, Roosevelt Ave. 50, 1050 Brussels, Belgium
[2] Interuniversity Institute of Bioinformatics in Brussels, Université Libre de Bruxelles, CP 263, Triumph Bld, 1050 Brussels, Belgium

Corresponding author: Rooman, Marianne (mrooman@ulb.ac.be)

Current Opinion in Structural Biology 2017, **42**:117–128

This review comes from a themed issue on **Proteins: bridging theory and experiment**

Edited by **Igor N Berezovsky** and **Ugo Bastolla**

http://dx.doi.org/10.1016/j.sbi.2016.12.007

S0959-440X/

Introduction

An important challenge in protein science consists in unraveling the mechanisms by which the heat and cold resistance of proteins is modulated in order for their host organism to adapt to extreme environmental conditions, with temperatures that range from about −20°C to over 120°C [1]. The understanding of these mechanisms has important practical applications in the context of the optimization of protein-based biotechnological and biopharmaceutical processes. This involves the rational design of proteins with modified thermal characteristics, and opens the way towards *de novo* design [2].

In the last decades a lot of efforts have been made in this direction (see [3–5] and references therein). The global picture that emerges is that there is no universal, unique, adaptive mechanism but rather an intricate combination of different factors, which frequently differs according to the protein or protein family and is thus highly difficult to disentangle. Moreover, due to the lack of direct and general methods of investigation, the results are often confused and sometimes contradictory, and thus only some general trends are definitely settled.

The present paper reviews what is known regarding the physical mechanisms at the molecular scale that proteins use to remain folded and functional in either hot or cold environments. It is not intended to be a full comprehensive review, but rather a concise point of view of the newest results, debated and contradictory hypotheses, and perspectives for reaching a deeper understanding of the field.

Protein thermal stability

Definitions

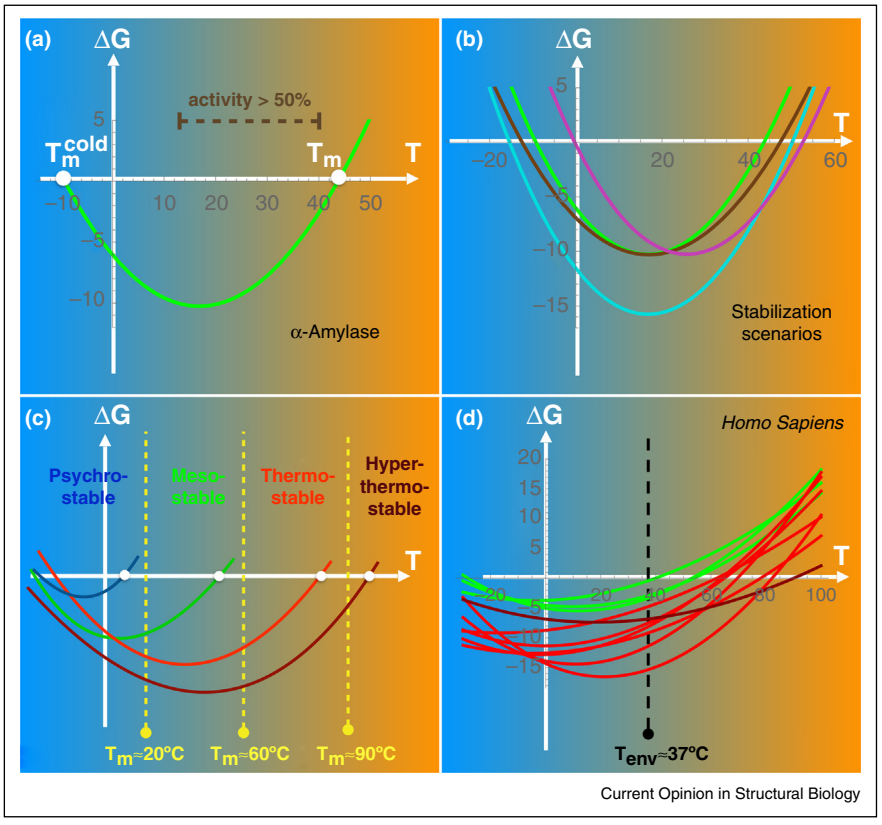
It can be generally assumed that structured proteins occur in two states, the folded and unfolded states, with the former being more populated in the temperature range $T_m^{cold} < T < T_m^{hot}$, with T_m^{cold} and T_m^{hot} the denaturation temperatures where the folding/unfolding transitions occur, whereas the latter is more populated outside this temperature range (Figure 1).

The heat denaturation temperature or melting temperature $T_m^{hot} \equiv T_m$ is commonly taken as the best descriptor of thermal resistance. A protein is considered as more thermally stable than another if its T_m is higher. It is in general biologically active up to this temperature, except if mutations in key sites prevent the enzymatic reaction, ligand binding or conformational change, in a nutshell, the proper functioning of the protein. The separation into psychrostable, mesostable, thermostable, and hyperthermostable proteins is performed on the basis of the T_m.

In contrast, the cold denaturation temperature T_m^{cold} is rarely measured since water usually freezes before this transition is reached. Moreover, even if a protein remains folded at low temperature, it is often inactive, usually due to a lack of flexibility. T_m^{cold} is thus not a good descriptor of cold adaptation, which has instead to be directly related to the activity at low T.

A frequent confusion is made between the thermal stability of a protein and the living or optimal growth temperature of its host organisms (OGT), and the latter is often wrongly taken as thermal stability descriptor. This leads inevitably to some misunderstanding since

Figure 1



Protein stability curves defined by ΔG as a function of the temperature T. **(a)** Experimentally characterized stability curve for the cold-adapted α-amylase from *Alteromonas haloplanktis* (AHA) (Uniprot code P29957, Enzymatic Commission number 3.2.1.1) [6]. The range in which the relative activity is larger than 50% is reported in the figure. **(b)** Different stabilization strategies to increase protein thermal stability with respect to AHA α-amylase (green curve): broadening of the stability curve by increasing the change in heat capacity upon folding (brown), shift of the entire curve towards more stable temperatures by increasing the temperature of maximum stability (magenta) or shift to more negative ΔGs by increasing the folding enthalpy (cyan). **(c)** Example of stability curves of psychrostable, mesostable, thermostable and hyperthermostable proteins that are defined according to their T_m value; note that the thresholds are conventional and are sometimes assigned to different values. **(d)** Experimentally derived stability curves for some proteins belonging to *Homo sapiens*, which hosts proteins with different thermal stability properties: from mesostable proteins with T_m close to the living temperature (37°C) to hyperthermostable proteins with T_m of more than 90°C [7].

the two quantities are only partially correlated: while a thermophilic organism only host thermostable proteins, a mesophilic organism can host both mesostable and thermostable proteins (Figure 1) while psychropholic organisms can in principle host all types of proteins.

Investigation methodologies

Current experimental and computational techniques do not capture a global picture of protein stability and adaptation, and yield a patchwork of results that are sometimes difficult to reconcile. They are summarized in Table 1 with their respective advantages and limitations.

Temperature dependence of the amino acid interactions

A proper analysis of protein thermal stability requires considering that the amino acid interactions are temperature dependent and thus that some are more stabilizing than others at higher or lower temperatures and *vice versa*. We review the interactions whose T-dependence has been discussed in the literature and summarize them schematically in Figure 2.

Hydrophobic effect and amino acid hydrophobicity

The hydrophobic effect constitutes the main driving force of protein folding and results from the tendency of hydrophobic amino acids to cluster together in order to avoid contact with water. Its T-dependence is directly connected to cold denaturation [8,9]. Indeed, whereas the hot denaturation is associated to a thermal increment of the conformational fluctuations, the cold transition is mainly related to the weakening of the hydrophobic effect [10•] that becomes unfavorable below a certain temperature compared to hydrophobe–water interactions.