

Locking out water at 100°C.

Jeremy C Smith.

Thermophilic proteins, present in organisms that live at high temperatures, denature at much higher temperatures compared to their mesophilic counterparts. How these proteins stand the heat has long been researched and is particularly interesting because homologous pairs of thermophilic and mesophilic proteins show a high degree of structural and sequence similarity. In early studies of thermophilic proteins the proportion of solvent-accessible charged residues was found to be increased at the expense of polar residues [1,2]. Further analyses confirmed this and also noted strengthening of hydrophobic cores by branched apolar residues [3]. At the same time, whether thermophilic proteins actually need to be more or less flexible than their mesophilic counterparts and the potential usefulness of surface loop deletion has been debated [4-7].

A relatively clear solution for thermostability seems to have been adopted by a particular hyperthermophilic protein, glutamine amidotransferase (GATase) from the archaeon, *Methanocaldococcus jannaschii* (MjGATase). This protein is post-translationally modified, converting Asn109 into a succinimidyl residue (SSN) that confers remarkable thermal stability on the protein, which resists unfolding up to 100 °C. In this issue Dongre et al [8] present a crystal structure of MjGATase and, using enhanced sampling molecular dynamics (MD) simulations, address the mechanism of its increased thermostability. The succinimide residue (SNN109) lies at the solvent exposed tip of a loop in the protein, and this is curious as SNN is notoriously prone to detrimental hydrolysis. However, Dongre et al show that in MjGATase SNN109 is protected from hydrolysis by electrostatic shielding by the side chain carboxylate group of its succeeding residue, Asp110 as well as through $n \rightarrow \pi^*$ interactions between SNN109 and its preceding residue, Glu108, both of which prevent water access to SNN, even in extreme heat.

So how does the SSN furnish stability? Well, the experiments and simulations show that the conformationally-restricted succinimide-containing segment forms a strong local conformational lock, even at the highest temperature examined through simulation, inducing the formation of an alpha-turn structure involving a 13-atom hydrogen bonded ring. This rigidity is shown to propagate through the protein, reaching the active site. Further, conservation of the succinimide-forming tripeptide sequence (E(N/D)(E/D)) in several archaeal GATases suggests an adaptation of this otherwise detrimental post-translational modification may have been an early evolutionary ploy for thermostability in the rude environments of hot springs and hydrothermal vents and on primitive Earth.

References

1. Cambillau, C. and Claverie, JM. Structural and genomic correlates of hyperthermostability. *J. Biol. Chem.* 275.42 (2000): 32383-32386.
2. Suhre, K. and Claverie JM. Genomic correlates of hyperthermostability, an update. *J. Biol. Chem.* 278.19 (2003): 17198-17202.
3. Kumar, S., and R. Nussinov. How do thermophilic proteins deal with heat? *Cellular and Mole. Life Sci. CMLS*58.9 (2001): 1216-1233.

4. Yu, H. and H. Huang. Engineering proteins for thermostability through rigidifying flexible sites. *Biotech. adv.*32.2 (2014): 308-315.
5. Meinhold, L., D. Clement, M. Tehei, R. Daniel, J.L. Finney, & J.C. Smith, (2008). Protein dynamics and stability: the distribution of atomic fluctuations in thermophilic and mesophilic dihydrofolate reductase derived using elastic incoherent neutron scattering. *Biophys. j*, 94(12), 4812-4818.
6. Liu, Z., S. Lemmonds, J. Huang, M. Tyagi, L. Hong, & N. Jain. (2018). Entropic contribution to enhanced thermal stability in the thermostable P450 CYP119. *Proc. Natl. Acad. Sci.* 115(43), E10049-E10058.
7. Agarwal, R., U.R. Shrestha, X.Q. Chu, L. Petridis and J.C. Smith. Mesophilic enzyme function at high temperature: molecular dynamics of hyperthermophilic and mesophilic pyrophosphatases. *Biophys. J.* 119 1142–150 (2020)
8. Dongre et al. *Biophys J.* This Issue.

This manuscript has been authored by UT-Battelle, LLC, under contract DE-AC05-00OR22725 with the US Department of Energy (DOE). The US government retains and the publisher, by accepting the article for publication, acknowledges that the US government retains a nonexclusive, paid-up, irrevocable, worldwide license to publish or reproduce the published form of this manuscript, or allow others to do so, for US government purposes. DOE will provide public access to these results of federally sponsored research in accordance with the DOE Public Access Plan (<http://energy.gov/downloads/doe-public-access-plan>).