Is the protein folding code truly understood? The curious (and exotic) case of fold switching proteins

Anand Srivastava

Molecular Biophysics Unit, Indian Institute of Science, Bangalore, KA 560012

Email: anand@iisc.ac.in

Functionally important metamorphic proteins, which can switch folds between different well-folded conformations, are now thought to be widespread. These protein sequences do not obey the classical folding dogma, and the underlying principles that drive their fold-switching behavior is poorly understood. In my talk, I will present two diverse approaches towards understanding the molecular grammar of fold switchers - one based on bioinformatics and other on thermodynamics principles. In the first part, I will present a new fragment-based classification algorithm from my group, named as Morpheus, which can annotate fold-switching proteins from sequence information across proteomes (https://doi.org/10.1101/2025.02.13.637956). Since Morpheus server takes in user defined sequences, it can also act a useful screening tool towards the de novo design and engineering of such proteins through further experimentation. However, rationale de novo design of fold switching proteins will require a thorough understanding of the molecular driving forces leading to their conformational propensities. In the second part of my talk, I will discuss our recent unpublished work on understanding the thermodynamic design principles that leads to fold-switching behavior in such proteins. As a test case, we considered a recently designed fold-switching protein [John Orban and co-authors, PNAS 2023, 120 (4), e2215418120] that transitions between a 3α fold and α/β fold upon changes in temperature. We performed a detailed thermodynamic analysis on this protein using an advanced and efficient free energy all-atom molecular simulation approach. We find that while 3α fold is stabilized at low temperatures by enthalpic contributions from favorable water-water and protein-water interactions, the transition to the α/β fold at high temperatures is driven by the gain of entropy from the release of ordered water molecules surrounding the 3α conformer into the bulk. Our study elucidates the importance of thinking in terms of entropy-based design in natural systems and provides a framework that can help in the design and engineering of future synthetic and functional metamorphic proteins.