ENTROPY AND WATER DYNAMICS IN ENZYMATIC POLYCYCLIZATION REACTIONS

Charlotte Kürten, Science for Life Laboratory, Stockholm – School of Biotechnology, Protein Engineering and Enzyme Design, KTH, Sweden
charlotte.kurten@scilifelab.se

Per-Olof Syrén, KTH Royal Institute of Technology – School of Chemical Science and Engineering, Sweden

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Polycyclic molecules have potent chemical and biological properties and are widely used in various industrial applications e.g. as flavoring compounds in the food industry or bioactive compounds in pharmaceutical industries. They are generated in complex polycyclization reactions that follow sophisticated and concerted reaction pathways associated with very high entropic costs originating from the loss of rotational freedom of the pre-folded substrate.

Enzymatic polycyclization of linear isoprene molecules by terpene cyclases generates a vast divergence in the chemical class of terpene molecules leading to new functionalities and bioactivities in all domains of life. Triterpene cyclases introduce two- to six ring structures into linear 30-carbon molecules, forming versatile scaffolds with potent activities or as substrates for further functionalization and modification reactions leading to the miscellaneous “terpenosome” of higher organisms.\(^1\)

Herein, computational design and biophysical analyses of the membrane-bound human oxidosqualene cyclase\(^2,3\) showed that enzymatic lanosterol production is driven by a favorable entropy of activation. In silico analyses revealed a tunnel network in hOSC that allows for water passage between the active site and the surrounding bulk solvent. By introducing rationally designed, tunnel perturbing, single point amino acid substitutions we could show that the thermodynamic profile of the enzymatic reaction is dependent of the tunnel and water network and thus can be altered by changing the tunnel pattern.

The importance of water dynamics in sophisticated biosynthetic machineries intrigued us to study the interplay of solvent and protein dynamics in triterpene cyclases. Differences in hOSC variants with obscured tunnel networks and disturbed thermodynamic signatures are studied with an LC-MS/MS based approach\(^4\) together with crystallographic structural determination of the membrane protein variants.

1 Miettinen, K. et al. The ancient CYP716 family is a major contributor to the diversification of eudicot triterpenoid biosynthesis. Nature Communications 8, 14153, doi:10.1038/ncomms14153 (2017).