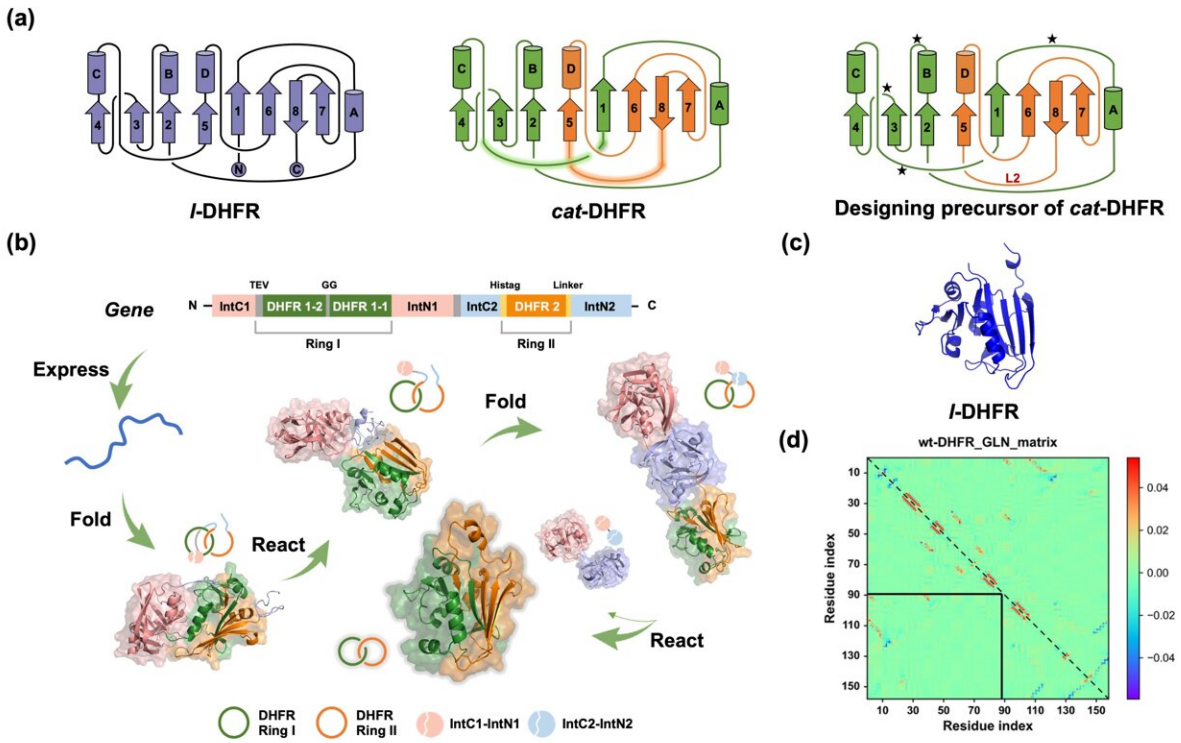
Artificial design and biosynthesis of a single-domain catenated dihydrofolate reductase

December 29 2023



(a) Protein topological diagram of *l*-DHFR (left) and *cat*-DHFR (middle), and the retrosynthetic analysis of *cat*-DHFR (right). Numbers 1–8 and letters A–D represent the β-sheet and α-helix, respectively, from N- to C- termini in consecutive order. The split site (residues 88 and 89) is located at the loop region between α-helix-C and β-sheet-5. The highlighted lines are the linkers generated when forming the *cat*-DHFR. The star denotes the possible position for ring I closure (at the same and opposite sides) and split-intein insertion. L2 is the linker newly introduced to ring II of *cat*-DHFR. (b) Scheme of the *cat*-DHFR biosynthesis process using programmed post-translation processing events.

DHFR1 is circularly permutated, and the corresponding sequences are denoted by DHFR1-1 and DHFR1-2. The TEV recognition site and a GG linker were inserted into ring I. The His-tag and a variable linker (together, they are L2) were inserted into ring II. (c) Structure prediction (https://robetta.bakerlab.org/) of *l*-DHFR. (d) The Gaussian linking number (GLN) matrix of wt-DHFR. It comprises GLN values between all neighboring residue pairs within the same chain. The sum of all the cells within the boxed sub-matrix corresponds to the GLN value between the two subchains, which provides a quantitative metric of the extent of their entanglement. Credit: Science China Press

This study was led by Prof. Wen-Bin Zhang (College of Chemistry and Molecular Engineering, Peking University & Beijing Academy of Artificial Intelligence) and Dr. Jing Fang (College of Chemistry and Molecular Engineering, Peking University). A single-domain protein catenane refers to two mechanically interlocked polypeptide rings that fold synergistically into a compact and integrated structure, which is extremely rare in nature.

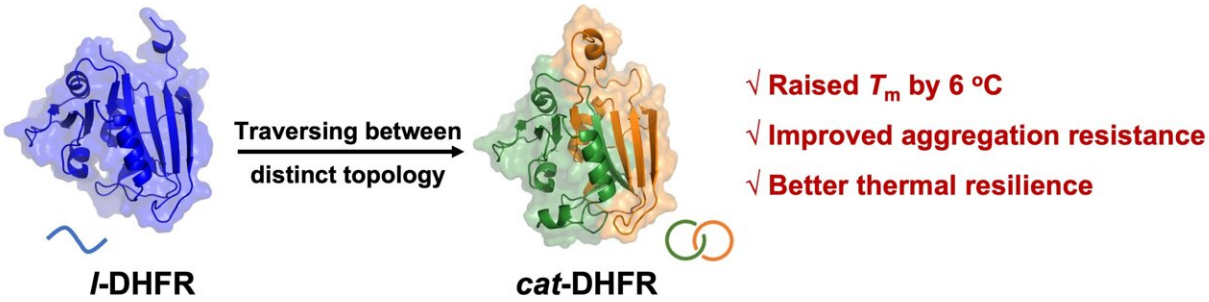
This design was achieved by rewiring the connectivity between secondary motifs to introduce artificial entanglement, and synthesis was readily accomplished through a series of programmed streamlined post-translational processing events in cells without any additional in vitro reactions.

The single-domain catenane *cat*-DHFR was thoroughly characterized. Evidence from combined SDS-PAGE, SEC, LC-MS, IMS-MS, and proteolytic digestion experiments unambiguously proved its topology. The *cat*-DHFR exhibits enhanced anti-aggregation properties and has a T_m that is 6 °C higher than the linear control.

Although the catalytic activity of *cat*-DHFR is reduced owing to its decreased affinity toward the substrate and cofactor, it has better

thermal resilience than *l*-DHFR. Even after incubation at 70 °C for 10 min, *cat*-DHFR retained over 70% of the catalytic activity, whereas the linear control lost almost all activity.

The research team anticipates that this method could be generally applicable to other single-domain proteins, including those with folds similar to DHFR or with completely different folds. The availability of these single-domain protein catenanes facilitates the elucidation of topological effects on structure–property relationships.



A single-domain protein catenane of DHFR was designed and directly synthesized in cellular, which raises the Tm by 6 °C relative to the linear control and also has improved aggregation resistance as well as enhanced thermal resilience. Credit: Science China Press

The results further imply that it is possible to map the current linear protein universe into single-domain protein catenanes with well-preserved functions and additional benefits, opening up new territory for protein molecules. Surpassing the linear paradigm of natural protein molecules, these topological proteins are multi-chain, multi-dimensional molecules with functional benefits of topology, rich design possibility, and excellent evolvability.

As a new class of protein molecules, they hold great potential for a broad range of applications, including, but not limited to, industrial enzymes, antibodies, cytokines, and biomaterials.

The study is published in the journal *National Science Review*.

More information: Jing Fang et al, A Single-domain Protein Catenane of Dihydrofolate Reductase, *National Science Review* (2023). DOI: 10.1093/nsr/nwad304

Provided by Science China Press