Hyperstable Serum Albumin Variants for Microbial Mass Production

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Overview

Serum albumins are essential in a wide variety of molecular and cellular applications, including in the medical, biotechnological and cultivated meat industries. Albumins, however, are currently sourced from animal serum, raising significant concerns about reproducibility, scalability, and ethics. To address these issues, Prof. Fleishman and his team have designed and validated improved and boiling-resistant serum albumin variants that can be efficiently produced by bacteria.

Background and Unmet Need

Albumins derived from animal or human plasma present ethical and reproducibility challenges (e.g., contamination risks, batch-to-batch variability, and animal welfare). Additionally, the growing demand for serum albumin in the cultivated meat industry far exceeds the current production capacity of recombinant proteins. While yeast-based production has been explored, it remains complex. In contrast, bacterial production offers advantages in terms of speed, cost, and efficiency, but the complex structure of albumin has precluded its heterologous production, until now.

The Solution

Computationally designed hyperstable variants of mammalian serum albumins readily expressed and purified from *E. coli.*

Technology Essence

Using the PROSS computational platform, stable designs of human and bovine serum albumins (HSA and BSA) were developed, exhibiting superior thermal stability compared to plasma-derived counterparts, of up to 40Â°C and even beyond the boiling point. The optimized designs were robustly expressed in *E. coli* in 100s of mg/l culture in standard lab settings. By contrast, the natural albumins did not express solubly. The engineered HSA and BSA variants were not toxic to HEK293T and hybridoma cells, carried typical small-molecule ligands of albumins and exhibited potent activity in restriction enzyme reactions, thus enabling applications in cell culture, and molecular biology (Figure 1).

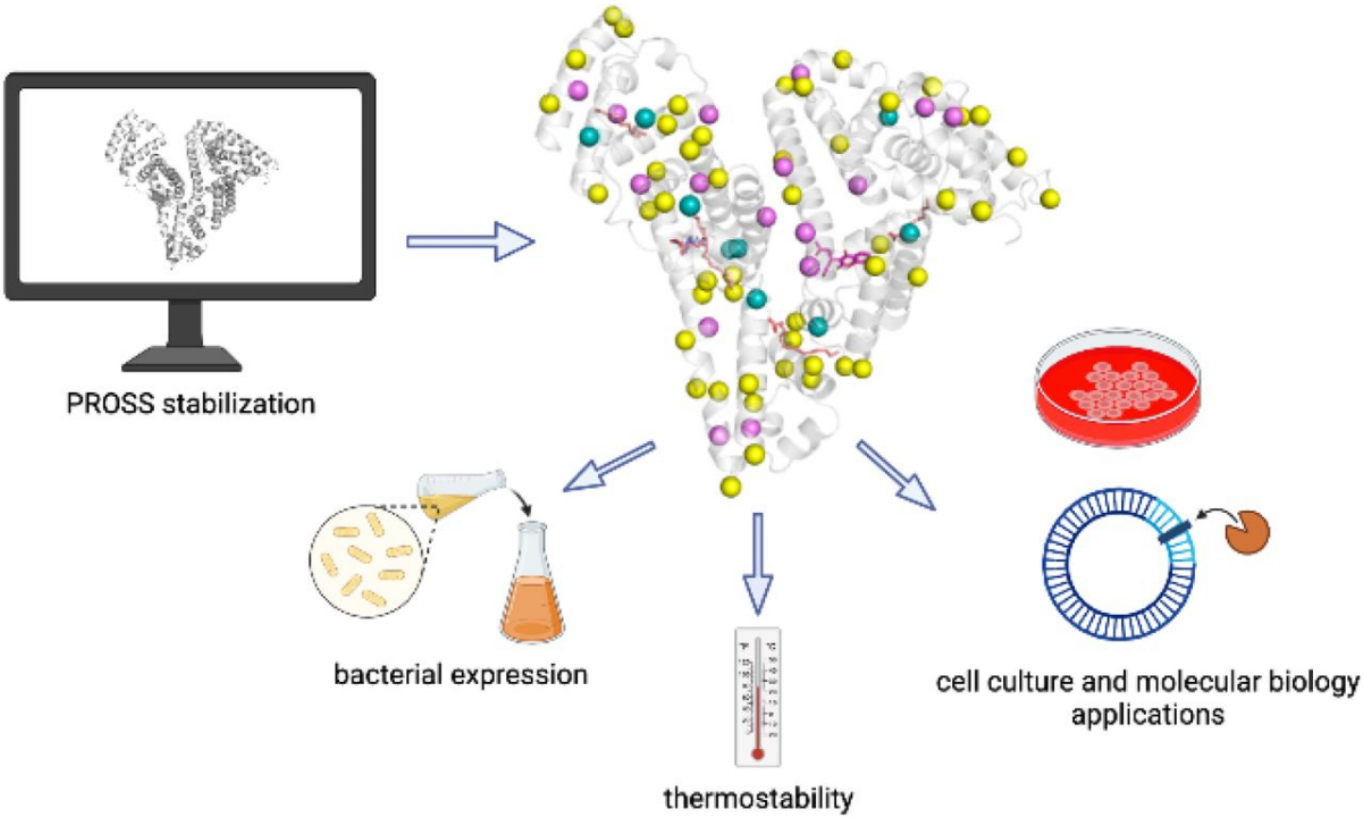

Figure 1. Stabilized HSA and BSA designs with improved thermal properties, generated through computational modeling for cell culture, and biotech applications.

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Applications and Advantages

- Fast and economical large-scale production of animal-free reagents
- Applicable to cultivated meat, cell culture and molecular biology applications
- Significantly higher thermal stability, enabling direct purification from bacteria by boiling

Development Status

All tested stabilized variants were highly expressed in *E. coli* and their functional properties were validated in cell culture and *in vitro* applications.

References

Khersonsky O, Goldsmith G, Zaretsky I, Rogotner S, Dym O, Unger T, Yona M, Fridmann-Sirkis Y, and Fleishman SJ. 2023. â€œStable Mammalian Serum Albumins Designed for Bacterial Expression.â€ *bioRxiv, 2023*. Â https://www.biorxiv.org/content/10.1101/2023.03.28.534334v2 [1]

Patent Status

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