Poster Presentation

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Design and structure determination of thermally stable adenylate kinase variants

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Thermally stable proteins are desirable for research and industrial purposes, but redesigning proteins for higher thermal stability can be challenging. A number of different techniques have been used to improve the thermal stability of proteins even though the extent of stability enhancement remains unpredictable and is often not significant. Here, we systematically tested the effects of multiple stabilization techniques on a single protein, thereby providing an integrated approach to protein thermal stabilization. Using mesophilic adenylate kinase as a model, we identified stabilizing mutations based on various stabilization techniques, and generated a series of adenylate kinase variants by introducing mutations both individually and collectively. The redesigned proteins displayed a range of increased thermal stabilities, the most stable of which was comparable to a naturally-evolved thermophilic homologue with more than a 25 degree increase in its thermal denaturation midpoint. We also solved crystal structures of three representative variants including the most stable variant, to confirm the structural basis for their increased stabilities. These results provide a unique opportunity for systematically analyzing the effectiveness and additivity of various stabilization mechanisms, and they represent a useful approach for improving protein stability by simultaneously optimizing global non-covalent interactions and local structural entropy.

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