Design, selection, and characterization of a split chorismate mutase.

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Details

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Abstract

Split proteins are versatile tools for detecting protein-protein interactions and studying protein folding. Here, we report a new, particularly small split enzyme, engineered from a thermostable chorismate mutase (CM). Upon dissecting the helical-bundle CM from Methanococcus jannaschii into a short N-terminal helix and a 3-helix segment and attaching an antiparallel leucine zipper dimerization domain to the individual fragments, we obtained a weakly active heterodimeric mutase. Using combinatorial mutagenesis and in vivo selection, we optimized the short linker sequences connecting the leucine zipper to the enzyme domain. One of the selected CMs was characterized in detail. It spontaneously assembles from the separately inactive fragments and exhibits wild-type like CM activity. Owing to the availability of a well characterized selection system, the simple 4-helix bundle topology, and the small size of the N-terminal helix, the heterodimeric CM could be a valuable scaffold for enzyme engineering efforts and as a split sensor for specifically oriented protein-protein interactions.

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