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Structural basis of substrate channeling in the PutA flavoenzyme

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Proline utilization A (PutA) is a high-hanging fruit of X-ray crystallography. PutA is a membrane-associated bifunctional flavoenzyme that catalyzes the 4-electron oxidation of proline to glutamate by the sequential activities of proline dehydrogenase and aldehyde dehydrogenase domains. PutAs are challenging crystallography targets because of their long polypeptide chain length (1000-1300 residues) and multidomain architecture. In this talk, I will present new crystal structures and SAXS analysis of two PutAs. Seven high resolution crystal structures of a 1004-residue minimalist PutA were determined using Hg SIRAS phasing, and the oligomeric state and quaternary structure were determined with SAXS [1]. The structures reveal an elaborate and dynamic tunnel system featuring a 75-Å long tunnel that links the two active sites. Also, a novel mechanism-based inactivation strategy allowed the trapping of the elusive PutA-quinone complex in the crystalline state. These structures provide insight into the mechanism of substrate channeling and how the enzyme changes conformation during the catalytic cycle. I will conclude by describing the first structure of a new type of PutA that contains an additional C-terminal domain of unknown function (CTDUF) that is not present in the smaller minimalist enzyme [2]. This larger PutA reveals an unexpectedly different structural solution to the problem of sequestering the reaction intermediate.

[1] *H. Singh, et al., PNAS, 2014, In press.,* ***[2]*** *Unpublished*

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