Seminars in Biotechnology BTEC 591 & BTEC 691

"Directed Evolution of a Thermophilic Cytochrome P450 Enzyme to Increase H2O2-Dependent Activity and Characterization of Novel Improved Variant"

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Dr. Sürmeli got her B.Sc. degree in the Department of Chemistry at Bilkent University in 2003. She got M.A. degree from Princeton University in 2005 and got her Ph.D. degree from Princeton University Department of Chemistry in 2009. She worked with Prof. John T. Groves at Princeton University, and she investigated the interaction mechanisms of metalloproteins with reactive oxygen and nitrogen molecules there. Then, as a postdoctoral researcher, she worked in the lab of Prof. Michael A. Marletta at the University of California. Afterward, she transferred to the Scripps Research Institute with the lab she worked. She studied the nitric oxide receptor soluble guanylyl cyclase (sGC) enzyme there. She joined the Department of Bioengineering of İzmir Institute of Technology in 2015. Her research areas include protein design and engineering, biocatalysts, and biochemistry.

Abstract

Biocatalysts are increasingly utilized in chemical synthesis due to their high level of regioselectivity and enantioselectivity. Cytochrome P450 (P450) enzymes are important biocatalysts due to their ability to hydroxylate unactivated carbon atoms using molecular oxygen. P450s catalyze reactions by using NAD(P)H as electron donor and require electron transfer proteins. P450s can also utilize H2O2 instead of NAD(P)H through the H2O2-shunt pathway. However, this reaction is inefficient. CYP119 is a thermophilic P450 from *Sulfolobus acidocaldarius.* Here, directed evolution was used on CYP119 to develop a novel enzyme with higher H2O2-dependent activity. Directed evolution is a powerful tool to create novel enzymes. Evolution of natural enzymes to achieve desired properties are performed in

iterative rounds of random mutagenesis followed by a screening/selection method. CYP119 mutant library was constructed via combinatorial active site saturation test (CAST), and screened for improved peroxidation activity using Amplex® Red as substrate. This lead to an improved novel double mutant (DM). The DM CYP119 was characterized, it showed 5 fold higher catalytic activity than WT CYP119 for Amplex® Red peroxidation reaction. Also, the biocatalytic epoxidation of styrene was also analyzed under optimized conditions. DM CYP119 demonstrated 2 fold higher catalytic activity than CYP119 for styrene epoxidation. Therefore, a novel mutant of CYP119 that shows higher H2O2-dependent activity was obtained through directed evolution and the novel variant was chemically and spectroscopically characterized.