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Structural insight into the enhanced pollutant-degrading capabilities of engineered biphenyl dioxygenases and wild type phthalate dioxygenase

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Biphenyl dioxygenase of *Burkholderia xenovorans* LB400 BphAE (LB400)) is a multicomponent Rieske-type oxygenase that catalyzes the dihydroxylation of biphenyl and many polychlorinated biphenyls (PCBs). The structural basis for the substrate specificity of the enzyme oxygenase component (BphAE (LB400)) is largely unknown. BphAE (p4), RR4 and II9, variants obtained through directed evolution, transform several chloro biphenyls, including more efficiently than BphAE (LB400). Here, we compare the structures of BphAE (LB400) and its variants and examine the biochemical properties of these enzymes. This study provides important insight about how Rieske-type oxygenases can expand substrate the range through mutations that increase the plasticity and/or mobility of protein segments lining the catalytic cavity. Additionally, phthalate dioxygenase system (PDOS) initiates the aerobic breakdown of phthalate by forming cis-dihydrodiol phthalate with consumption of NADH and O_2. Here we present the structural insights into phthalate dioxygenase (PDO) from *Cupriavidus metallidurans*. The crystallographic structure of PDO and PDO-phthalate complex determined to 1.8 A˚ and 2.2 A˚ respectively, revealed an overall unique fold and unusual dioxygenation position of 3, 4 compared to other gram negative bacterial strains. We have depicted that catalytic domain features the largest substrate binding cavity characterized so far within oxygenase family, where part of cavity is lined with polar residues for interaction with phthalate.

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