**MS06-1-3 Identifying the key determinants of enzyme specificity in two stereo-complementary Class II pyruvate aldolases of bacterial origin**
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**I. Justo [1], S. Marsden [2], U. Hanefeld [2], I. Bento [3]**
**[1]EMBL - Hamburg (Germany), [2]Technische Universiteit Delft - Delft (Netherlands), [3]EMBL-Hamburg - Hamburg (Germany)**

**Abstract**
Class II pyruvate aldolases are enzymes capable of catalyzing the formation of new carbon-carbon bonds in a stereospecific and reversible manner, having lately attracted great attention as potential biocatalysts. In their enzymatic mechanism, these enzymes use a divalent metal cation as a cofactor and are highly specific for the nucleophile donor substrate. Some enzymes are able to take besides pyruvate, hydroxy- and fluoro- pyruvate as a donor substrate [1]. Regarding the acceptor aldehyde substrate, these hydroxy-ketoacid aldolases accept a diverse range of acceptor aldehydes. With the goal of identifying key residues that underlie the enzymatic mechanism and the stereospecificity of such enzymes, the structural and enzymatic characterization of two different hydroxyketoacid aldolases, namely SwHKA from Sphingomonas wittichii RW1 (3S,4S selective) [1] and BpHKA Burkholderia phytofirmans (3S,4R selective) [1] that show opposite stereoselectivity, was performed. Based on several high-resolution crystal structures of native and mutant enzymes, soaked with substrates that were obtained for both SwHKA [2] and BpHKA enzymes, and on the enzymatic characterization of such enzymes, we were able to identify a new resting state for SwHKA and key residues in the active site of both SwHKA and BpHKA responsible for the stabilization and for the correct orientation of the acceptor aldehyde for catalysis.

**References**
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