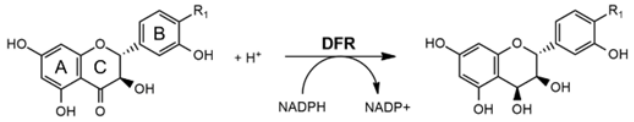
Exploring the substrate Specificity of Dihydroflavonol-4-Reductase.

DAVID Lucas;[A] **HASELMAIR-GOSCH Christian**;[B] **HALBWIRTH Heidi;** [B] **ANTONCZAK Serge** [*,A]

A) Institut de Chimie de Nice, Université-Côte d'Azur-CNRS, 28 avenue Valrose, Nice, France; B) Institute of Chemical, Environmental and Bioscience Engineering, Technische Universität Wien, Getreidemarkt 9, 1060 Vienna, Austria.

Serge.Antonczak@univ-cotedazur.fr

Dihydroflavonol 4-reductase (DFR) is a key enzyme in the biosynthesis of anthocyanins and catalyzes the reduction of dihydroflavonols to leucoanthocyanidins (see scheme 1). Some DFRs can convert dihydrokaempferol (DHK), dihydroquercetin (DHQ) and dihydromyricetin (DHM) irrespective of their B-ring hydroxylation pattern, whereas others show distinct substrate specificity. Differences apparently come from subtle mutations of the amino acid sequence. Despite an abundance of studies on DFR substrate specificity, there is still no systematic understanding of how the specificity is determined at the molecular level. This work addresses the DFR substrate specificity and structure-function relationship with respect to the B-ring hydroxylation pattern of the substrates.



Scheme 1. Reaction Mechanism catalyzed by Dihydroflavonol-4-Reductase Enzyme

In parallel of experimental DFR assays [1], Molecular Dynamics simulations are carried out on the substrate/enzyme complex in order to decipher the most important interactions that could improve or exclude an efficient enzymatic reaction, depending of the substrate. So far, the role of several catalytic amino acids involved in the reaction is well known[2], but the exact impact of amino-acids of the stabilizing pocket or of the second shell around the active site is not fully described (see Figure 1). Here, we will present our first investigations on their role using Molecular Dynamics protocols.

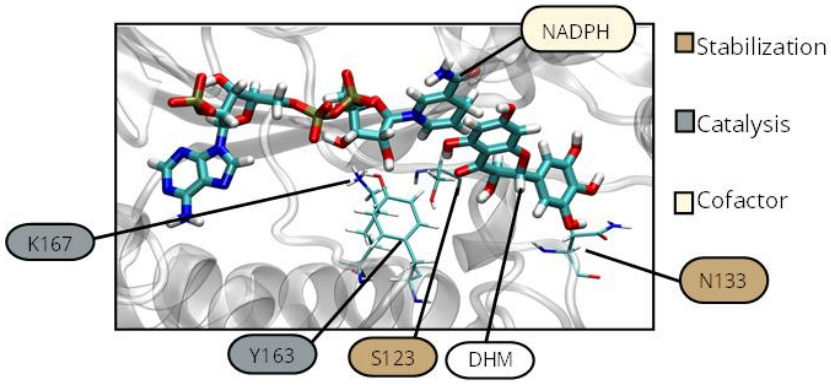


Figure 1. Dihydroflavonol-4-Reductase Active Site with Dihydromyricetin substrate

Keywords: Molecular Dynamics, Substrate Specificity, Dihydroflavonol-4-Reductase.

[1] C. Haselmair-Gosch, et al.. Frontiers in Plant Science 9 (2018) 149.

[2] J. Diharce, E. Bignon, S. Fiorucci, S. Antonczak. E. ChemBioChem, 23(3) (2022)