



INVESTMENTS IN EDUCATION DEVELOPMENT

CZ.1.07/2.3.00/30.0037

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AFFIDAVIT

I confirm that the research article "Fluorescence polarization analysis of binding kinetics of HaloTag ligands to haloalkane dehalogenase enzymes with modified access tunnels" contains results of the research supported during the implementation of the project „Employment of Best Young Scientists for International Cooperation Empowerment“ (POSTDOC II.), reg. number CZ.1.07/2.3.00/30.0037.

Brno, *8.10.2015*

Shubhangi Kaushik

Shubhangi Kaushik
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FLUORESCENCE POLARIZATION ANALYSIS OF BINDING KINETICS OF HALOTAG LIGANDS TO HALOALKANE DEHALOGENASE ENZYMES WITH MODIFIED ACCESS TUNNELS

Shubhangi Kaushik, Jiri Damborsky, Radka Chaloupkova

Loschmidt Laboratories, Department of Experimental Biology and Research Centre for Toxic Compounds in the Environment RECETOX, Masaryk University, Brno, Czech Republic

Quantitative analysis of biomolecular interactions can contribute to fundamental investigation of proteins and can provide information about their ability to accommodate various ligands. Fluorescence polarization (FP) is a method for rapid and non-destructive quantification of the association of small fluorescent ligands with a larger biomolecules in a real time [1]. Here we describe FP-based quantitative analysis of binding kinetics of selected HaloTag ligands into haloalkane dehalogenase enzymes. Haloalkane dehalogenases have wide range of applications in bioremediation, biosensing of subsurface pollutants, biosynthesis of optically pure chemicals, cellular imaging and protein analysis. Active sites of these enzymes are deeply buried in the protein core and connected with the surrounding solvent by several access tunnels [2]. Modification of protein tunnels by mutagenesis has emerged as a novel strategy to alter properties such as activity, specificity or selectivity of enzymes with buried active sites [3-4]. Binding behavior of a set of biochemically characterized variants of haloalkane dehalogenase DhaA and LinB with modified access tunnels were investigated. Our results revealed that FP method can sense the differences in binding kinetics of tested ligands with various haloalkane dehalogenases based on the nature of introduced mutations in their access tunnels. The differences in binding kinetics were detected even when a single point mutation was introduced in the access tunnel. Moreover, the binding kinetics was found to be influenced by the type of fluorescent ligand employed during the binding reaction. The method has a good potential for analysis of accessibility of tunnels and active sites in enzymes forming covalent intermediates and for medium-throughput screening of enzyme libraries to identify mutant candidates with optimal tunnel geometry. The site-saturation mutagenesis library of haloalkane dehalogenase DhaA31 [5] was constructed and screened using FP method to identify the variants displaying improved binding kinetics. The results of the screening and characterization of hits will be presented at the conference.

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