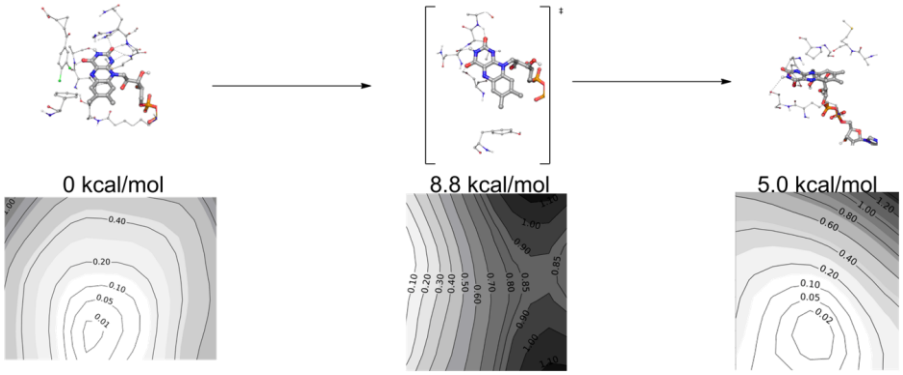
Umbrella sampling identification of the elusive ´*out*´ conformational state of kynurenine 3-monooxygenase

Yılmaz Özkılıç[a], Matthias Stein[a]

[a]*Molecular Simulations and Design Group, Max Planck Institute for Dynamics of Complex Technical Systems, Magdeburg, Germany*

Kynurenine 3-monooxygenase (KMO) is a member of the class A monooxygenase family which is characterized by the lack of a binding pocket for nicotinamide adenine dinucleotide phosphate (NADPH). It reduces the coenzyme flavin adenine dinucleotide (FAD) during the reductive half reaction. KMO hydroxylates L-kynurenine (L-Kyn) and releases the product 3-hydroxykynurenine (3-HK), which itself and its following compounds (3-hydroxyanthranilate (3-HanA) and quinolinate (Quin)) cause neurodegeneration [1]. L-Kyn is also a substrate to kynurenine aminotransferase (KAT) that converts it to the neuroprotective compound kynurenic acid (KynA) [2]. Decreasing the Quin/KynA ratio by inhibiting KMO is regarded as a possible way of treating neurodegeneration. Such inhibition would allow the treatment of central nervous system damages. Several successful inhibitors were developed over the years, but it was soon realized [3] that most of the inhibitors themselves cause the formation of neurodegenerative hydrogen peroxide (H_2O_2).



Molecular dynamics simulations of KMO and the inhibitor–KMO complexes were carried out using umbrella sampling to explore the free energy surface of the conversion from the ´*in*´ to the ´*out*´ conformational state change of the coenzyme FAD. In addition to the apo form of the enzyme, both a non-substrate effector inhibitor and a competitive inhibitor containing initial structures were utilized. A relative evaluation that allows the comparison of computationally obtained results with the experimental results is presented. This information is used to obtain the relative free energy change, e.g. the barrier associated with the conformational change and a model of the elusive ´*out*´ conformational state that was speculated but not observed experimentally. Ligand-residue interactions along the conformational change were determined to identify the influence of the effector.

[1] D. Zwilling, S.-Y. Huang, K. V. Sathyasaikumar, F. M. Notarangelo, P. Guidetti, H.-Q. Wu, J. Lee, J. Truong, Y. Andrews-Zwilling, E. W. Hsieh, others. *Cell*, **2011**, *145*, 863–874.
[2] T. W. Stone. *Trends Pharmacol. Sci.,* **2000**, *21*, 149–154.
[3] R. S. Phillips, E. C. Iradukunda, T. Hughes, J. P. Bowen. *Front. mol. biosci.* **2019**, *6*, 3.