Allosteric inhibitor Doramapimod can bind to human p38α MAP Kinase even when the activation loop is in the DFG-*in* state

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P38α MAP kinases are involved in key cellular processes, such as proliferation, apoptosis and differentiation. The flexible activation loop plays a key role in protein function and contains a highly conserved DFG (Asp-Phe-Gly) motif. In the DFG-*in* state the motif is involved in coordination of Mg^{2+} ion required for the ATP binding. In the alternative DFG-*out* state Phe169 implements conformation which overlaps with the ATP binding area. It was shown that blocking of the activation loop in the DFG-*out* conformation by an inhibitor can prevent the ATP binding and capture the enzyme in the catalytically inactive state [1, 2]. The ligand BIRB796, known as Doramapimod, is one of the most efficient human p38αMAPK inhibitors. Crystallographic studies have shown that Doramapimod binds to the allosteric site which becomes fully accessible to the solvent only when conformation of the activation loop changes to the DFG-*out* [3]. This prevents the return of the activation loop to the DFG-*in* conformation and inactivates the enzyme. Nevertheless, it remains unclear how the initial binding of the inhibitor occurs and whether the DFG-*out* state is required for this event. Molecular modeling has been implemented to study binding of Doramapimod by the human p38αMAPK. Molecular docking has identified position of Doramapimod binding on the surface of p38αMAPK when the activation loop is in the DFG-*in* conformation, which has RMSD of around 4Å compared to crystallographic complex of Doramapimod in p38αMAPK in the DFG-*out* conformation. The morpholine cycle of the ligand is accommodated in the ATP-binding pocket. Hydrogen bonds are formed between the ligand and the backbone and sidechain groups of Lys53, Glu71, Met109, and Asp168. The tert-butyl group of Doramapimod is accommodated in a hydrophobic pocket located above Phe169 whose sidechain is oriented towards a hydrophobic core. Molecular dynamics further implemented to study the enzyme-inhibitor complex during 100 ns at 373K has shown that the orientation

of the morpholine cycle of the ligand generally remains unchanged. In contrast to that the tert-butyl group of Doramapimod penetrates its way underneath the protein surface towards a more deeply located hydrophobic core by pushing out the sidechain of Phe169 within the first 20 ns of the simulation. As a result, the orientation of the inhibitor significantly changes and after 100 ns of simulation it becomes identical to the crystallographic complex. To conclude, molecular modeling has shown that BIRB796 (Doramapimod) can bind to a complementary allosteric pocket not only in the DFG-*out* state of the human p38α MAPK, but even in its DFG-*in* state what is followed by conformational changes of the activation loop that finally improve accommodation of the inhibitor.

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1. Filomia, F., De Rienzo, F., Menziani, M. C. (2010). Insights into MAPK p38α DFG flip mechanism by accelerated molecular dynamics. *Bioorg. Med. Chem.*, **18(18):** 6805-6812.;

2. Frembgen-Kesner, T., Elcock, A. H. (2006). Computational sampling of a cryptic drug binding site in a protein receptor: explicit solvent molecular dynamics and inhibitor docking to p38 MAP kinase. *J. Mol. Biol.*, **359(1):** 202-214.

3. Pargellis, C., et al. (2002). Inhibition of p38 MAP kinase by utilizing a novel allosteric binding site. *Nat. Struct. Mol. Biol.*, **9(4)**: 268-272.

4. Voevodin, V. V., et al. (2012). Practice of "Lomonosov" supercomputer. *Open Systems J*, **7:** 36-39.