The Role of PIF-Pocket in modulation of PDK1 Dynamics

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PDK1 is a "master" protein kinase in insulin and growth factor signaling. Phospholipid secondary messengers trigger the activation of a complex protein kinase network, in which PDK1 is responsible for the activation of many different protein kinases. In order to be activated, the cytosolic substrates first need to be phosphorylated at a conserved hydrophobic motif at their C-terminus. This specific sequence binds to the allosteric site on PDK1, termed as PIF-Pocket (for *PDK1 interacting fragment*), and is located at the N-terminal lobe of the kinase domain [1]. The specific binding event enables PDK1 be fully active and phosphorylate the substrate [2]. *Engel et al.* and *Stroba et al.* showed that PIF-Pocket is a druggable site and developed allosteric modulators of PDK1 [3, 4]. The main advantage of these modulators is that they are more specific than ATP competitive compounds.

The PIF-Pocket in almost all crystal structures represents a conformation which corresponds to the active state of the kinase. Even though there is an inactive crystal structure of the enzyme, the pocket is not suitable to accommodate ligands such as in active crystal structures. Therefore we have performed MD simulations of PDK1 in water and in methanol to establish a good understanding of how sampling of PDK1 conformations is coupled to altered pocket geometries. The simulations sampled various states of PDK1 between active and inactive conformations, and thus of the PIF-Pocket as well. Starting from the complex of PDK1 and ATP, we found that the PIF-Pocket shows a considerable degree of dynamics. To distinguish active conformations of PDK1 from the non-active ones we established a filtering protocol. We have performed docking experiments using experimentally proven activators and inhibitors.

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