Why targeting the inactive conformation of protein kinases with inhibitors leads to efficient therapies?

[†]*Ralitza Boubeva,* [†]*Andrea Cristiani,* [†]*Lucile Pernot,* [†]*Remo Perozzo,* [¥]*Francesco Luigi Gervasio,* [¥]*Ludovico Sutto,* [¥]*Marco D'Abramo,* [†]*Leonardo Scapozza*

[†]*Pharmaceutical Biochemistry Group, School of Pharmaceutical Sciences, Université de Genève, Quai E.Ansermet, 30 1211 Genève, SWITZERLAND*
[¥]*Centro Nacional de Investigaciones Oncológicas. C/ Melchor Fernández Almagro, 3, E-28029 Madrid (SPAIN)*

Keywords : mutation, crystal structure, ITC, molecular dynamics, catalysis, kinetics, activity test

c-Src tyrosine kinase is a plastic enzyme which can assume different conformations based on the activation state. The conformational transition is influenced by hydrophobic interfaces and "spines" within the kinase domain.

We found that L317I mutation in H1-H2 interface permits the recovery of nM activity of an inactive conformation marker as Imatinib. This showed that a single amino acid is able to modify the conformational propensity.

We solved the apo-form and Imatinib bound crystal structures of c-Src L317I mutant that revealed a peculiar active like conformation for the apo-structure and inactive conformation for the complexed protein. In line with the active-like conformation found by crystallography thermodynamic measurements with ITC revealed the same K_d values of Imatinib for both L317I and WT protein. These results suggest that the mutation modify the dynamic of the protein rather than stabilizing the inactive conformation.

To shed light on the dynamical behavior of the protein we performed large scale MD and metadynamics simulations of WT and mutated proteins. The MD clearly indicates that the rate of the flip of the DFG motif (a crucial and conserved element in catalysis) between *in* and *out* conformation is favored by the mutation L317I.

We measured the kinetics of both c-Src L317I and WT understanding the role of the ADP produced during the kinase activity. The product inhibition effect of ADP is clearly reduced in the mutant compared to WT protein supporting the hypothesis that the DFG-flip is essential for unbinding the product during the catalytic cycle.

Both kinetics measurements and molecular dynamic simulations support the possibility of a formation of an inactive-like conformation within the catalytic cycle of the kinase domain.