

# Computational Biophysics *Junior Group*

## Summary

Since biomolecules are by no means static, specific conformational changes often connect catalytically active and inactive conformations. Deregulation of activation dynamics is linked to numerous diseases including cancer. Our goal is to understand the atomistic details of the underlying dynamics as well as linking specific oncogenic mutations to changes in the activation mechanism. This will enable us to link genotypes to phenotypes as well as structure to function and help with the design of more selective and effective drugs.

## Strategic Goals

- Analyse the activation dynamics of proteins related to cancer
- Understand the role of induced folding and conformational selection in drug binding
- Develop advanced algorithms to increase the time and length scales of simulations

## Francesco L. Gervasio *Junior Group Leader (since February)*

Francesco L. Gervasio was trained in the field of Physical Chemistry and Spectroscopy in the Molecular Spectroscopy Laboratory and the European Laboratory for Non-linear Spectroscopy at the *Università di Firenze*, Italy, where he received his MD in 1997.

Upon completing his civil service duties, he started a PhD in Computational Chemistry at *Università di Firenze*, studies which he continued at the International School for Advanced Studies in Trieste at the department of Statistical and Biological Physics. He received his PhD in Chemistry in 2002 and that same year he joined the Swiss National Supercomputer Centre as a Junior Scientist.

In 2004 he joined the Group of M. Parrinello – one of the most eminent scientists in the field of computational chemistry, as a Post-doctoral Fellow at the *Eidgenössische Technische Hochschule* (ETH) in Zurich, Switzerland. In 2006 he was promoted to “*Oberassistent*” (a role similar to Assistant Professor) in computational chemistry at the ETH, Zurich. From 2006 – 2009, he was appointed as Professor at the prestigious *Scuola Normale di Pisa*, Italy.

Gervasio joined the CNIO as a Junior Group Leader in February 2009. He is an expert in molecular modelling and simulations and has developed effective algorithms to study large-scale dynamics in proteins and to predict the structure-activity relationship of drug-like molecules. He has authored 42 scientific papers in international journals which have been cited 860 times thus far. His h-index is 18.

He received a Fellowship Award in Drug Design in 2002, participated in a Royal Society International Joint Project in 2006 – 2007, and is collaborating with Sanofi-Aventis SA.





**Staff scientist:** Marco D'Abramo (since November). **Post-doctoral fellows:** Jaroslaw Juraszek (since June), Ludovico Sutto (since June). **Graduate student:** Ilaria Mereu (since October).

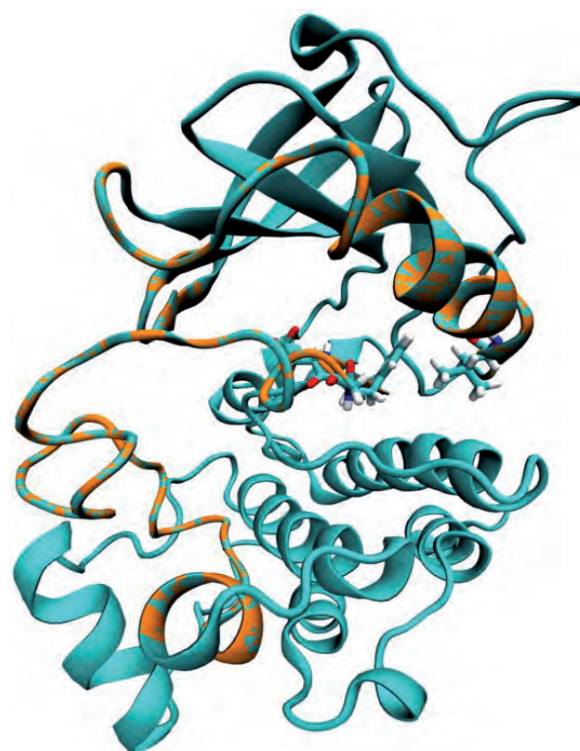
## Highlights

We are trying to understand the detailed mechanism(s) underlying the conformational plasticity of c-Src, a kinase that is involved in the oncogenic process of several tumours. Imatinib, an inactive-conformation-specific inhibitor, can be used for this purpose as an indirect marker of the conformation adopted by the c-Src kinase domain. Imatinib strongly binds to the DFG-out conformation of Abl kinase, while it binds poorly to the highly homologous c-Src.

Our working hypothesis is that this difference is due to the different propensity of the two kinases to adopt the DFG-out conformation. To prove this hypothesis – in collaboration with the Pharmacology Department at the University of Geneva – we studied the differences in the amino-acid sequences of c-Src and Abl. These two kinases differ by a few amino-acids in the DFG region. By mutating these amino acids one by one in c-Src to the corresponding Abl amino-acids, we have singled out one amino acid that can shift the conformational balance of c-Src towards the inactive Abl/c-Kit-like conformation.

We used MD complemented by metadynamics to understand how this single point mutation can affect the DFG flip. Our 1 $\mu$ s long simulations have shown that the mutation destabilises a cluster of hydrophobic residues. This cluster is located in the DFG region and in wild-type c-Src it stabilises the DFG-in conformation. This evidence has been corroborated by thermal unfolding experiments.

We are now running biased simulations of the DFG flip in both wild type and mutant c-Src. The preliminary results are consistent with a lower barrier to the flip and a more stable DFG-out conformation. In collaboration with the CNIO NMR Unit we have also expressed both wild type and mutant kinases in minimal medium to validate our computational findings against NMR data. If confirmed, our results could explain why the potent anticancer drug Imatinib does not bind c-Src.



**Figure:** Three dimensional cartoon representation of the c-Src kinase domain. The residues involved in the hydrophobic contacts stabilising the DFG motif in the active conformation are represented as sticks.

## Publications

Berteotti A, Cavalli A, Branduardi D, Gervasio FL, Recanatini M, Parrinello M (2009). Protein conformational transitions: the closure mechanism of a kinase explored by atomistic simulations. *J Am Chem Soc* 131, 244-250.

Cucinotta C, Kosa M, Melchiorre P, Cavalli A, Gervasio FL (2009). Bifunctional catalysis by natural cinchona alkaloids: a mechanism explained. *Chemistry* 15, 7913-7921.

Masetti M, Cavalli A, Recanatini M, Gervasio FL (2009). Exploring complex protein-ligand recognition mechanisms with coarse metadynamics. *J Phys Chem B* 113, 4807-4816.