# USE OF MOLECULAR DOCKING TO HIGHLIGHT THE MECHANISM OF ACTIVATORS AND INHIBITORS OF FARNESYL PROTEIN TRANSFERASE

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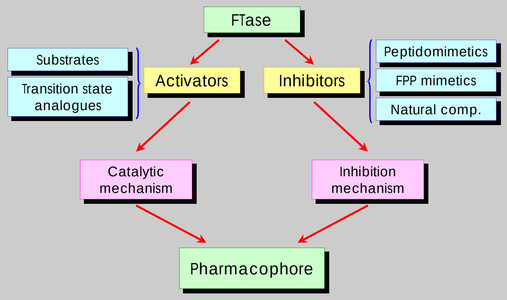
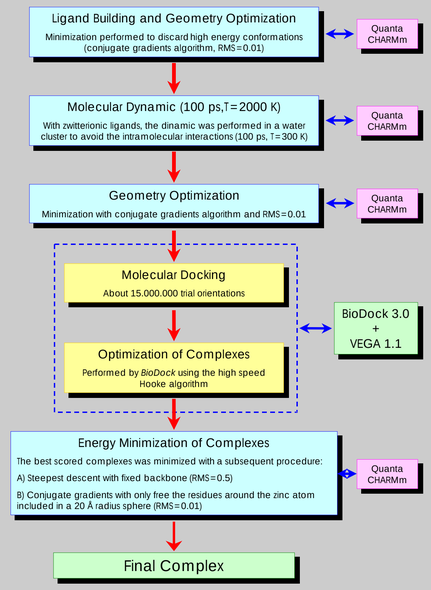
## INTRODUCTION AND RESEARCH AIMS

*Farnesyl protein transferase* (FTase) catalyzes the transfer of a farnesyl group from *farnesyl diphosphate* (FPP) to a specific cysteine residue of a substrate protein through covalent attachment[1,2].
This post-translational modification is involved in membrane association due to the enhanced hydrophobicity of the protein. This modification process has been identified in numerous proteins, including Ras proteins. Ras proteins play a crucial role in the signal transduction and cell division. Recently, there has been widespread interest in studying protein prenylation since the mutant forms of Ras proteins have been detected in 30% of human cancers. Thus, the design of FTase inhibitors is currently a major area of research. Knowledge about the active site environment of FTase is important for designing new inhibitors of the enzyme.



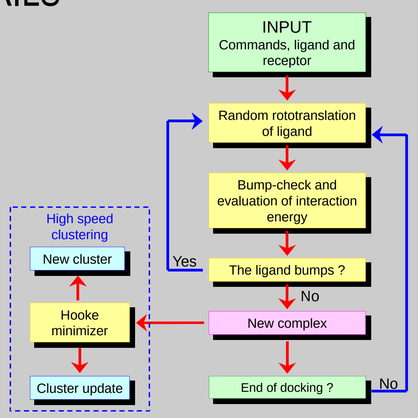

Recently the crystal structure of rat FTase was resolved at 2.25 Å resolution[2]. This protein is an heterodimer consisting of two subunits and the secondary structure of both the α and β subunits appears largely composed of α-helices. A single zinc ion, involved in catalysis[4,5], is located in the junction between the hydrophilic surface of β subunit and α hydrophobic deep cleft of β subunit. The zinc ion is coordinated by the β subunit residues *Asp-297, Cys-299, His-362* and a water molecule[2].
Cross-linking studies indicate that the binding sites for both protein and FPP lay on the β subunit[8]. The location for the two substrates can be inferred from the presence of two clefts that differ for their surface properties. One cleft is hydrophilic, being lined by charged residues and interacts with the CAAX peptide. The other cleft, orthogonal to this peptide binding site, is hydrophobic, being lined by aromatic residues and it is considered the site of FPP binding[6].
Experimental evidences show that CAAX motifs exhibit alternate binding modes and some CAAX peptides serve as FTase inhibitors and suggest that the enzymatic mechanism would be made up of two distinct phases.

## COMPUTATIONAL DETAILS



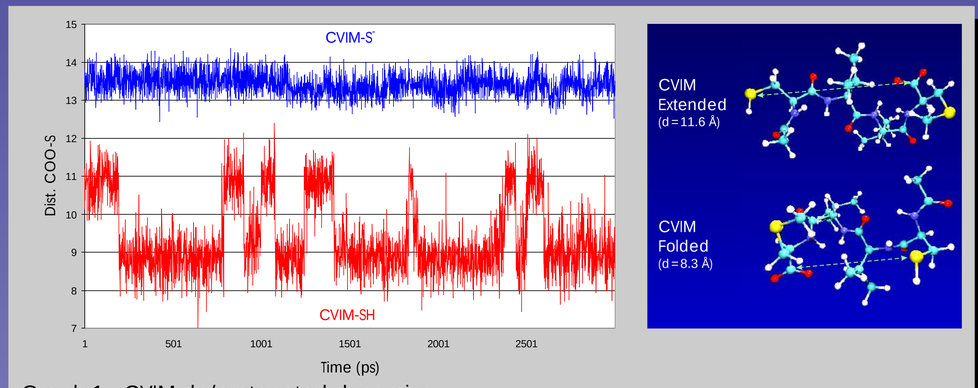

### Chart 1 - Computational steps

All molecular mechanics and dynamics calculation are performed with *Quanta/CHARMm* package (MSI Inc., Burlington, MA, USA).
The docking procedure is based on the stochastic algorithm implemented in *BioDock 3.0* software[9,10].
*VEGA 1.1* is a program specially developed to interface BioDock with some molecular software packages. In this tool, have been also implemented some features that are useful to analyze, display and manage the 3D structures of molecules.




### Chart 2 - BioDock schematics

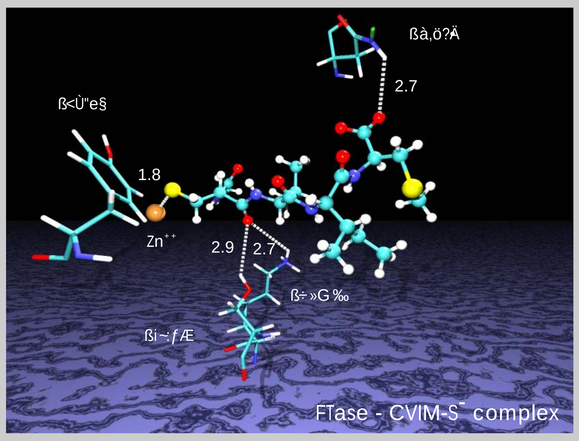
*BioDock* is the docking software developed in our laboratory, which is able to produce evaluate and classify a very high number of complexes between two interaction partners in a fast and efficient way. In the present version, both the compound are kept fixed. For each compound, about 15.000.000 complexes have been screened using energetical and sterical criteria implemented in BioDock. This software use a stochastic approach to generate the orientations of the ligand-receptor complex. In this way, many orientations can be similar without introducing new information about the binding site. Thus, each acceptable complex is clustered and only the best representative orientations are stored.







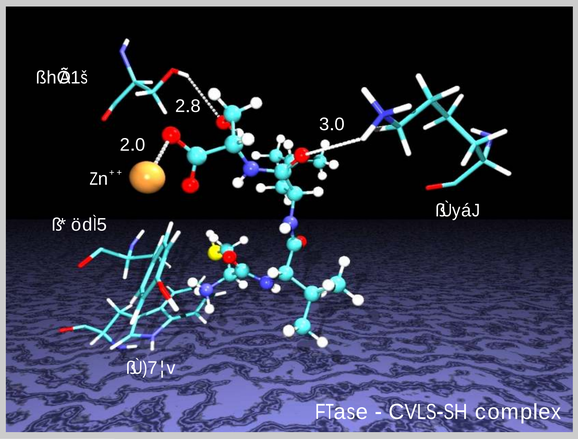
Graph 1 - CVIM de/protonated dynamics
Plot of the distance between C-term carboxylic group and cysteinic sulfur, during the two molecular dynamics.

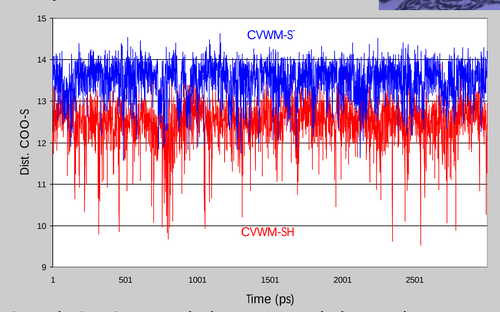



### Conformational Analysis of tetraptide derivatives

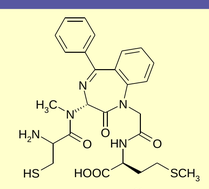
For each activator (CVLS, CVIM) and inhibitor (CVWM, CVFM) a long molecular dynamic simulation was performed (Time = 3 ns, T = 300 K). In order to highlight the influence of -SH cysteinic group in the conformation stabilization, an analogue dynamic of deprotonated tetrapeptides was also performed.
All activators with protonated sulfur, can exist in both folded and extended conformations. If the cysteinic residue is deprotonated, the electrostatic repulsion between sulfur and C-term carboxylate locks the extended conformation and the sulfur anion interacts with zinc ion (see Graph 1).

The dynamics of two tetrapeptidic inhibitors show that the only allowed conformation is extended not depending on the protonation of cysteinic residue (see Graph 2).
This information suggests with most probability that the extended conformation, common for all tetrapaptides, is involved in a recognition phase whereas the close conformation is involved in catalytic step. Thus, the substrate peptide performs a conformational change (from extended to folded conformation) during the enzymatic reaction. The CVWM e CVFM peptides can't perform this conformational transition and thereby aren't farnesylated.
The protonation of sulfur group allows folded conformations that interact with FTase realizing a ion pair between carboxylic moiety and zinc ion.

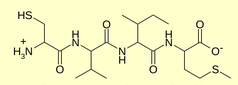


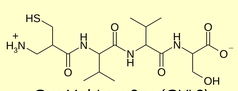



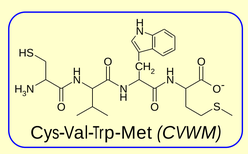

Graph 2 - CVWM de/protonated dynamics
Plot of the distance between C-term carboxylic group and cysteinic sulfur, during the two molecular dynamics. This peptide can never assume the folded conformation.

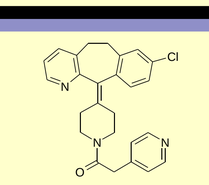


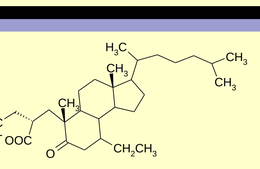

FTase recognizes the $CA_1A_2X$ motif[1] at the C-term of substrate, where C is the cysteine to which the prenyl group is attached, $A_1$ and $A_2$ are aliphatic residues, and X specifies which prenyl group is attached. If $A_2$ is aromatic (Phe, Tyr, Trp) the substrate binds the FTase but isn't farnesylated[3].

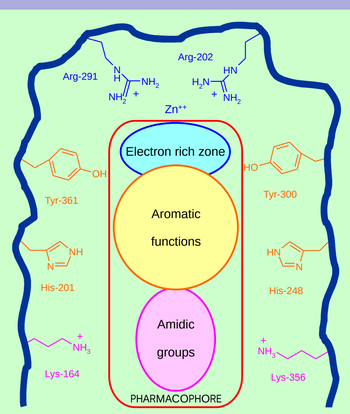


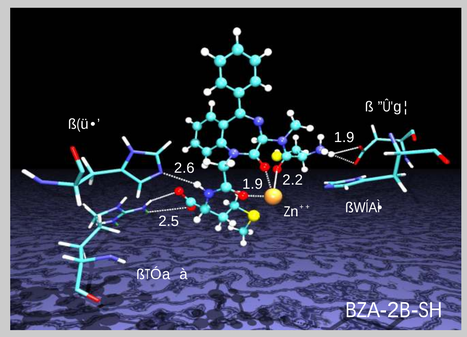



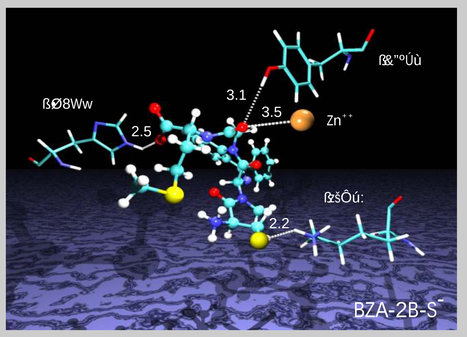



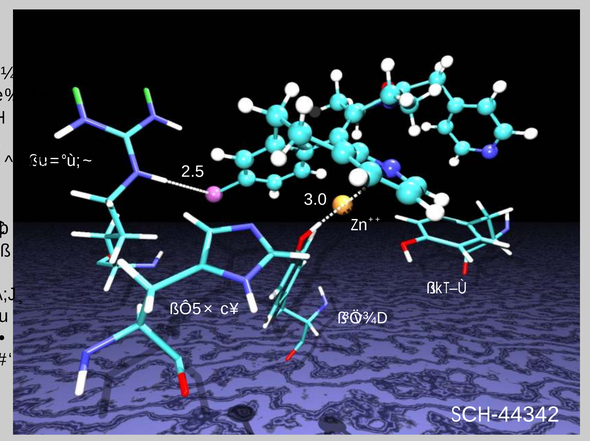



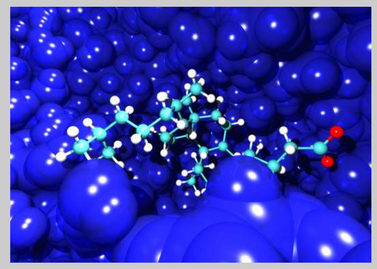



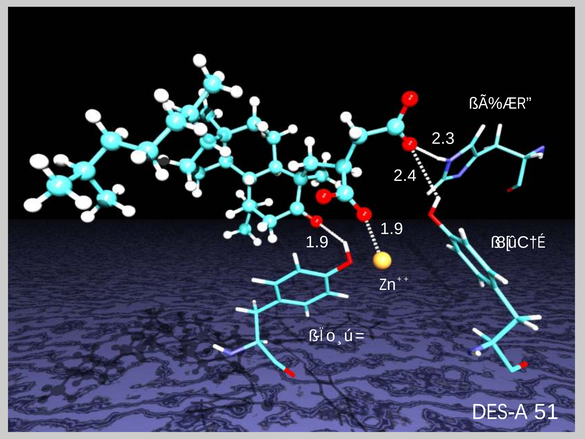




FTase - BZA-2B complexes
This inhibitor binds the enzyme miming the folded conformation of peptidic activators. As tetrapeptides, BZA-2B can interact with sulfur group protonated or ionized and the best fitting is obtained with protonated form. Indeed, the sulfur anion is never able to coordinate the zinc ion.






FTase - DES-A 51 complex
The obtained results show that the gonanic system imitates the tetrapeptide backbone in folded conformation. It's interesting to observe that this moiety don't perform specific interactions but realizes an optimal fitting with the enzyme.

## DISCUSSION

The docking studies of over 100 FTase ligands allow to highlight the most important features required to bind the enzyme:

- An electron rich zone, equivalent to cysteine sulfur, that interacts with an electron poor zone defined by $Zn^{++}$, Arg-202β and Arg-291β.
- Aromatic moieties, equivalent to $A_1$ and $A_2$ residues, that are able to make π-π interactions with Tyr-361β, Tyr-300β, His-248β and His-201β.
- At least the presence of carboxyamidic group useful for h-bonds with Lys-164α and Lys-356β.

This model was successfully used to study and optimize the series of isotiazolic inhibitors synthetized by *M. Valle* in the laboratories of *Istituto di Chimica Organica - Università di Milano*.

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