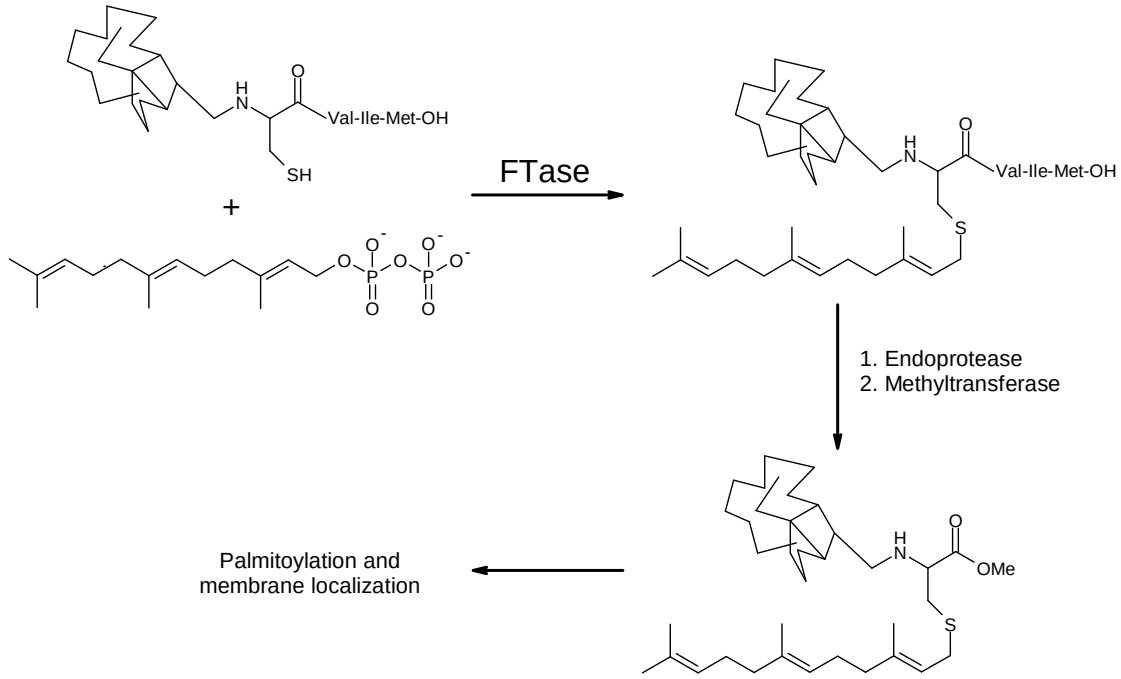
USE OF MOLECULAR DOCKING TO HIGHLIGHT THE MECHANISM OF ACTIVATORS AND INHIBITORS OF FARNESYL PROTEIN TRANSFERASE.

Alessandro Pedretti
Istituto di Chimica Farmaceutica e Tossicologica, Università degli Studi di Milano
Dottorato di Ricerca in Chimica del Farmaco (XIII Ciclo)

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 enzyme, like as *geranylgeranyl-transferase*, recognizes a common *CA_1A_2X* amino acid sequence[1] located at the C-terminus of substrate proteins. In the CA_1A_2X motif, C is the cysteine residue to which the prenyl group is attached, A_1 and A_2 are aliphatic amino acids, and X is the carboxyl terminus that specifies which prenyl group is attached. If X is Ala, Cys, Gln, Met, or Ser, the protein is a substrate for FTase and is farnesylated. If X is Leu or Phe, the protein is geranylgeranylated. This post-translational modification is believed to be involved in membrane association due to the enhanced hydrophobicity of the protein upon farnesylation. This modification process has been identified in numerous proteins located in eukaryotic organisms, including Ras proteins. Ras proteins play a crucial role in the signal transduction pathway that leads to cell division. It has been shown that farnesylation of Ras is necessary for proper functioning in cell signaling. Recently, there has been widespread interest in studying protein prenylation since Ras oncoproteins are farnesylated and mutant forms of Ras have been detected in 30% of human cancers. Since the farnesylation of oncogenic Ras proteins is required for cellular transformation, preventing the farnesylation process may be a possible approach for cancer chemotherapy. This prevention may be achieved through developing specific inhibitors of FTase, the enzyme that catalyzes the farnesylation of Ras; the design of such FTase inhibitors is currently a major area of research. Knowledge about the active site environment of FTase is important for designing new, potent 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 48 kD (α) and 46 kD (β) subunits and the secondary structure of both the α and β subunit appear largely composed of α-helices. A single zinc ion, involved in catalysis[3,4], is located at junction between the hydrophilic surface of β subunit and a hydrophobic deep cleft of β subunit. The zinc 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 reside on the β subunit[5]. The location for the two substrates can be inferred from the presence of two clefts that differ in their surface properties. One cleft is

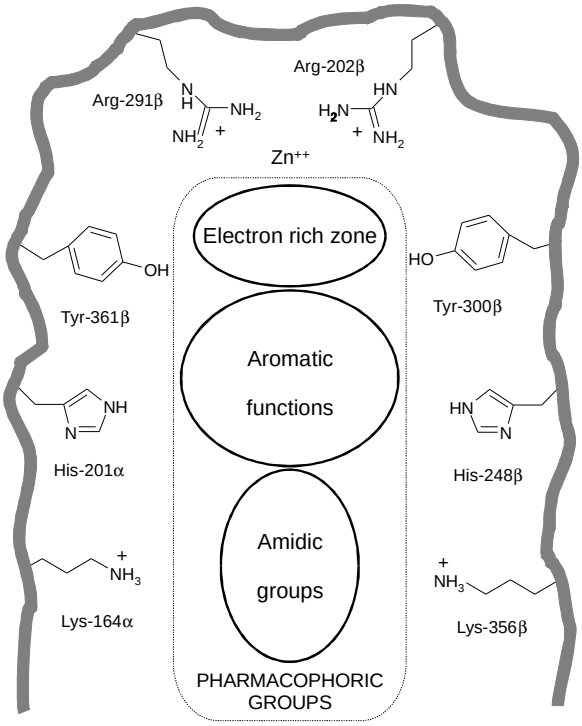
hydrophilic, being lined with charged residues and interacts with the CAAX peptide. The other cleft, orthogonal to this peptide binding site, is hydrophobic, being lined with aromatic residues and is considered the site of FPP binding[5].



The first step of RAS protein posttranslational modification is the covalent linkage between FPP, derived by classical isoprenoid biosynthesis pathway, and cysteine residue of CAAX[6]. This step is followed by cleavage of the last three aminoacids. The identification of the protein responsible for the proteolytic cleavage offers another target for blocking RAS activation. The final posttranslational modification, prior to membrane anchorage, is the methylation of the carboxyl group of prenylated cysteine. S-adenosyl-L-methionine (AdoMet) is the methyl donor. Inhibitors against the methyltransferase has been reported. The next modification is the palmitoylation of cysteine residue located upstream of farnesylated cysteine. This modification increases the binding affinity to the cell membrane, although not be essential.

In the present study, some well known and some potential inhibitors have been docked to the FTase crystal structure in order to highlight possible interaction differences and to define a reasonable pharmacophoric model. The analyzed compounds can be referred to benzodiazepinic, triciclic and isoprenoid analogues. Some natural derivatives was also studied[7].

The computational approach is based on *BioDock/VEGA*[8] software, developed in our laboratory to perform a stocastic docking of small ligands into biomacromolecules with known 3D structure. For each examined compound, about 10.000.000 complexes have been screened and clustered using energetical and sterical criteria implemented in BioDock. For the most interesting complexes, a constrained molecular minimization was performed with Quanta/CHARMm[9] package. Using the combined approach with BioDock and



Quanta/CHARMm simulations, one can obtained good results in a few time. Indeed, the BioDock tool provide a lot of possible orientations and performs a preliminary optimization in a limited time, keeping fixed all atoms, while the Quanta/CHARMm simulations analyze the mutual flexibility for the best complex only.

The analysis of the common aminoacidic residues of FTase involved in the interaction of all examined ligand with the enzyme, allows to find the substructures that are mainly needed for the inhibitor activity. This knowledge can be useful to design new drugs with more potent pharmacological activity.

References

1. L. Moores, M. D. Schaber et al., "Sequence dependence of protein isoprenylation", J. Biol. Chem, 266 14603-14610, 1991.
2. H.W. Park, S. R. Boduluri, P.J.Casey et al., "Crystal structure of protein farnesyl transferase at 2.25 Å resolution", Science, 275 1800-1805, 1997.
3. J. Chen, D. A. Andress et al., "cDNA cloning and expression of the peptide binding beta subunit of rat p21ras farnesyltransferase, the counterpart of yeast DPR1/RAM1", Cell, 66 327-334, 1991.
4. J. Chen, D. A. Andress et al., "Cloning and espression of a cDNA encoding the alpha subunit of rat p21ras farnesyltransferase", Proc. Nat. Acad. Sci. USA, 88 11368-11372, 1991.
5. W. Fu, J. F. Moomaw et al., "Identification of a cysteine residue essential for activity of protein farnesyltransferase", J. Biol. Chem., 271 28541-28548, 1996.
6. R. Lowy, B. M.Willumsen et al., "Function and regulation of RAS", Ann. Rev. Biochem., 62 851-891, 1993.
7. D.M. Leonard, "RAS farnesyltransferase: a new therapeutic target", J. Med. Chem., 40 2971-2990, 1997 and references cited herein.
8. A. Pedretti, "Nuovo metodo per il docking automatico di ligandi con macromolecole a struttura 3D nota", degree thesis, Milan University, 1995.
9. Quanta/CHARMm, MSI Inc., Burlington, MA, USA.