MODELLING OF THE INTERACTIONS OF SOME INHIBITORS WITH THE FARNESYL PROTEIN TRANSFERASE BY BIODOCK - STOCHASTIC APPROACH TO THE AUTOMATED DOCKING OF LIGANDS TO BIOMACROMOLECULES

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INTRODUCTION

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].

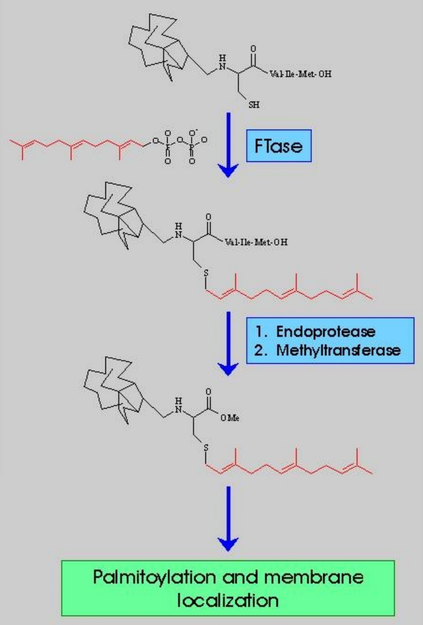
This enzyme, like as *geranylgeranyl-transferase*, recognizes a common CA$_1$A$_2$X amino acid sequence[3] located at the C-terminus of substrate proteins. In the CA$_1$A$_2$X motif, C is the cysteine residue to which the prenyl group is attached, A$_1$ and A$_2$ are aliphatic amino acids, and X is the 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[4]. This protein is an heterodimer consisting of 48 kD (alpha) and 46 kD (beta) subunits[5] and the secondary structure of both the and subunit appear largely composed of -helices[4]. A single zinc ion, involved in catalysis, is located at junction between the hydrophilic surface of subunit and a hydrophobic deep cleft of subunit. The zinc is coordinated by the subunit residues *Asp-297, Cys-299, His-362* and a water molecule[5].

Cross-linking studies indicate that the binding sites for both protein and FPP reside on the subunit. 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 bindig site, is hydrophobic, being lined with aromatic residues and is considered the site of FPP binding[4].

RAS PROTEIN POSTTRANSLATIONAL MODIFICATIONS

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.



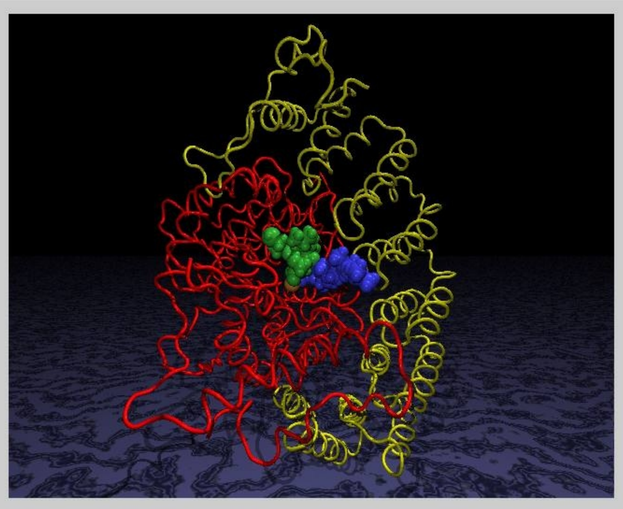


Figure 1 - FTase-FPP-H-RAS complex

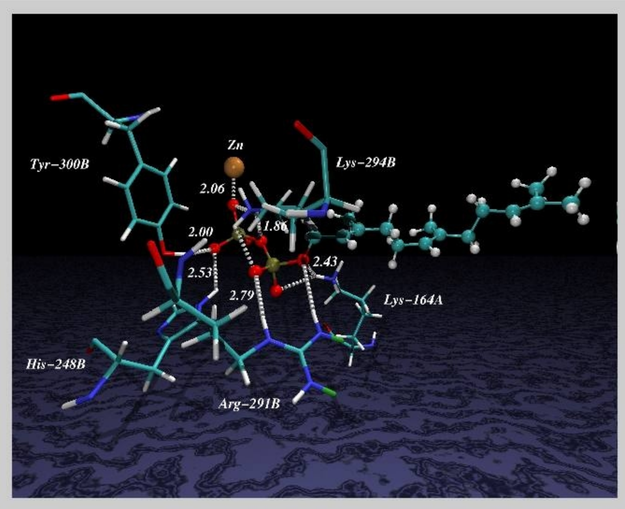
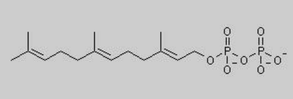


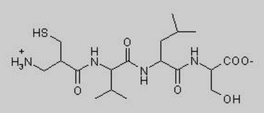
Figure 2 - FTase-FPP complex

H-RAS AND FPP DOCKING WITH FTASE

Both ligands (FPP and H-RAS-1 carboxy tetrapeptide CVLS terminus) was docked separately with FTase following the computational procedure described in methodology section (Chart 1) and final orientations was assembled in a ternary complex (FPP + CVLS + FTase). This complex was subjected to initial minimization (conjugate gradients, RMS = 0.01, backbone fixed) to discard high energy interactions, followed by 200 ps molecular dynamic (T = 300K, Newton equation integrated every two fs, backbone fixed). The lowest energy frame obtained by final 100 ps of MD trajectory was finally optimized using same conditions of preliminary minimization.



Farnesyl pyrophosphate (FPP)



H-RAS-1 CVLS segment

MTEYKLVVV GAGGVGKSAL TIQLIQNHFV DEYDPTIEDS YRKQVVIDGE
TCLLDILDTA GQEEYSAMRD QYMRTGEGFL CVFAINNTKS FEDIHQYREQ
IKRVKDSDDV PMVLVGNKCD LAARTVESRQ AQDLARSYGI PYIETSAKTR
QGVEDAFYTL KLNPPDESGP GCMSCK**CVLS**

H-RAS-1 Aminoacidic sequence

Main interactions:
- Electrostatic and coordination interaction between zinc ion and pyrophosphate oxygens negatively charged.
- The pyrophosphate group interacts also with Arg-291B, Lys-294B and Lys-164A.

The FPP aliphatic chain, even if not shown, is placed in FTase hydrophobic pocket.

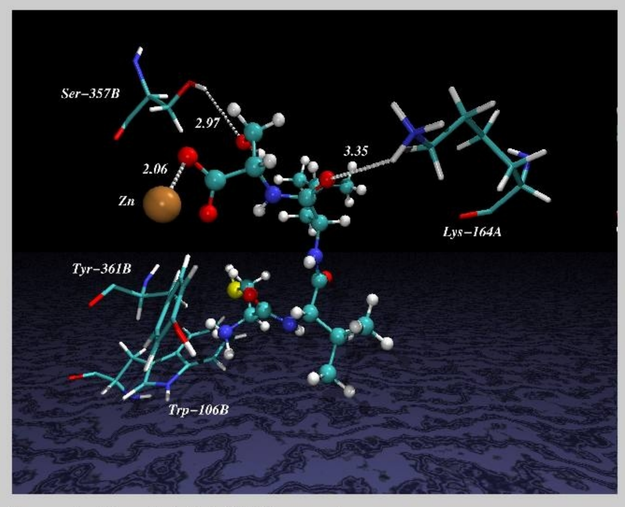
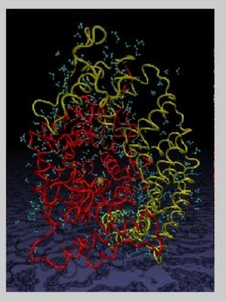


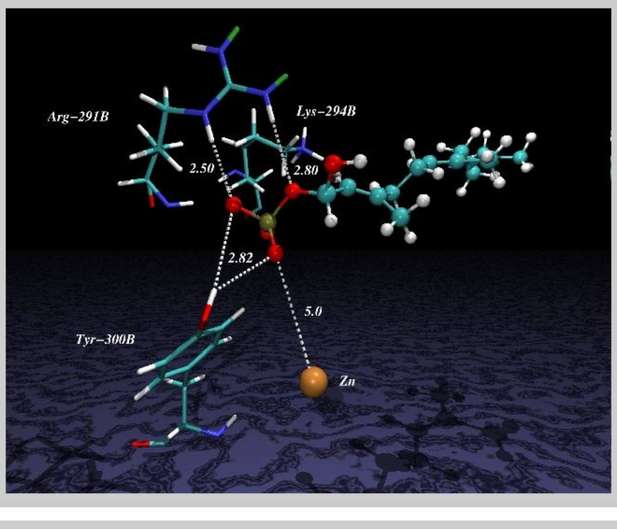
Figure 3 - FTase-H-RAS-1 (CVLS) complex

Main interactions:
- Electrostatic and coordination interaction between zinc ion and C-term carboxyl group.
- The N-term ammonic group is inserted in a pocket lined by aromatic residues. Obviously, this interaction is not present in natural complex FTase-H-RAS-1, but its interesting to perform a comparison with RAS-mimetic inhibitors.
- Electrostatic interaction between Lys-164A and carbonyl group.

Figure 4
FTase crystal structure

The crystal structure of rat farnesyl transferase, resolved by Park et al.[7], is available to *Protein Data Bank*[8] with the identification code 1FT1. This figure shows that the crystal contains a very large amount of water molecules. In all simulations performed during this docking study, these water molecules are kept.





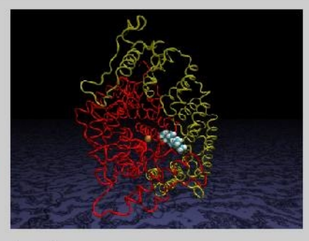
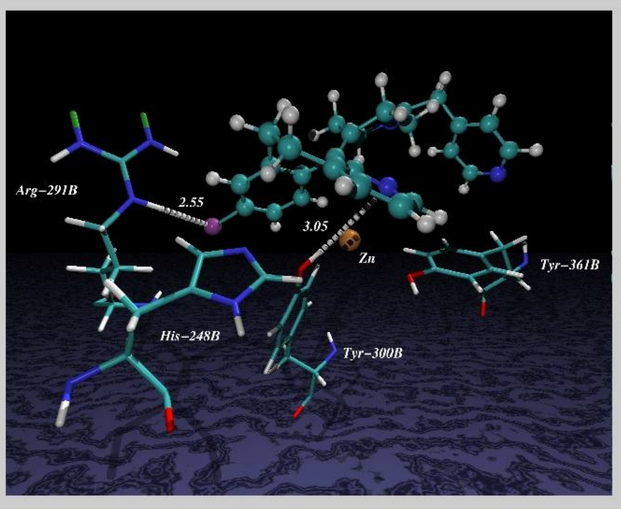


Figure5
FTase-(S) FPOH complex

Both enatiomers were docked into FTase, but only S isomer shows a interesting interaction.
Main interactions:
- Electrostatic interactions between Arg-291B and phosphate moiety.
- H-bonds between Tyr-300B and phosphate oxygens and between Lys294-B and hydroxyl group.

The change of pirophosphate group (present in natural FPP substrate) with phosphate don't allow the coordination with zinc ion.



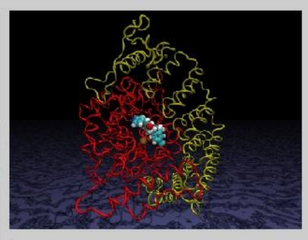
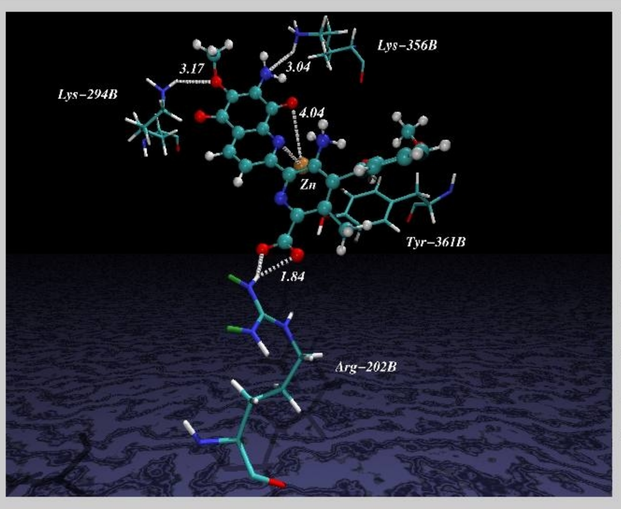


Figure 7
FTase-SCH-44342 complex

Main interactions:
- The characteristic conformation of the tricyclic system allows strong - interaction with several aromatic residues (His-248B, Tyr-300B and Tyr-361B).
- H-bonds interaction between Tyr-300B and nitrogen atom of pyridinic ring and between Arg-291B and chlorine atom.



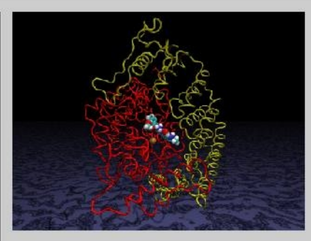
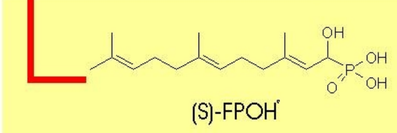
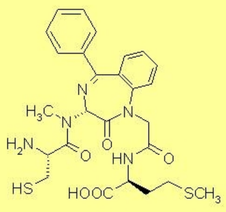


Figure 9
FTase-10'-Desmethoxystrepto-nigrin complex

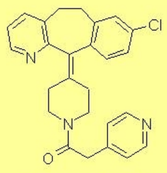
Main interactions:
- Electrostatic and coordination interaction of the zinc ion with carbonyl and nitrogen atom of 5,8-dihydro-5,8-diketo-quinolin fragment.
- H-bonds and electrostatic interaction between Arg-202B and carboxyl group.
- H-bonds between Lys-294B and methoxylic group and between Lys-356B and aminic group.



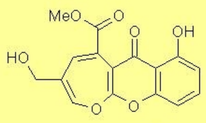
(S)-FPOH[9]



BZA2-B[10]

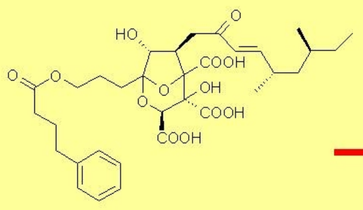


SCH-44342[11]



Fusidienol[12]

10'-Desmethoxystreptonigrin[13]



Zaragozic acid[14]

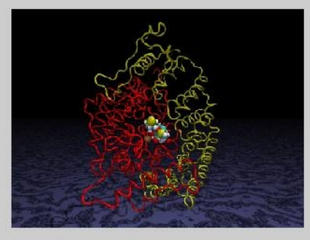
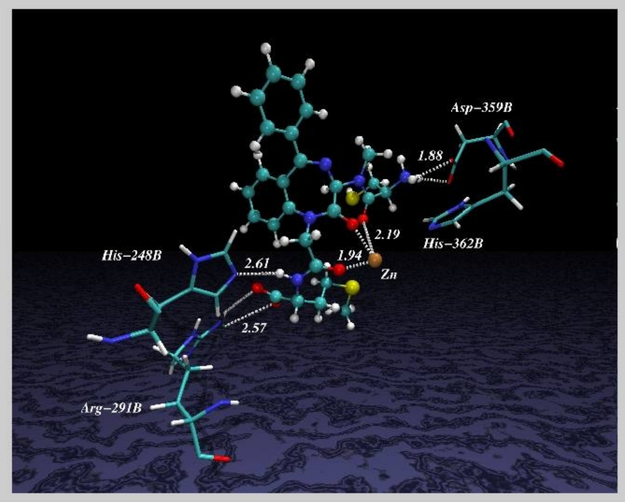


Figure 6
FTase-BZA-2B complex

Main interactions:
- Electrostatic and coordination interaction between zinc ion and carbonyl groups of the amidic moiety.
- H-bonds and electrostatic interaction between Arg-291B and carboxyl group.
- H-bonds and electrostatic interaction between Asp-359B and ammonic ion.



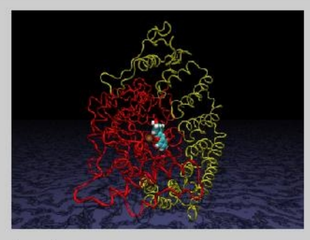
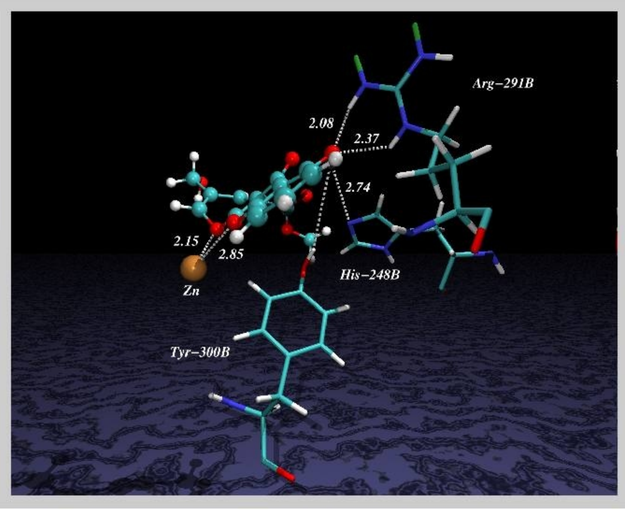


Figure 8
FTase-Fusidienol complex

Main interactions:
- Coordination interaction between zinc ion and cyclic ether oxygens.
- H-bonds and electrostatic interaction between Arg-291B and carbonyl groups.



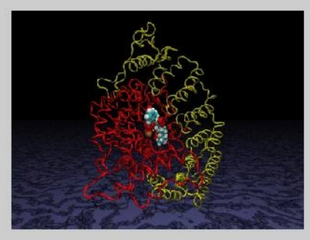
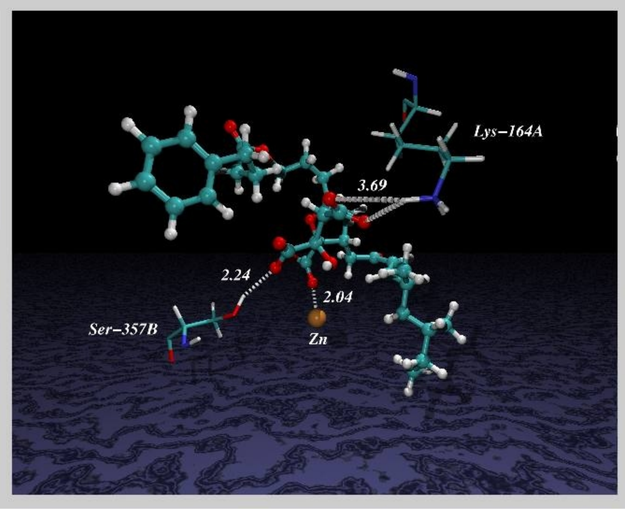


Figure 10
FTase-Zaragizic acid complex

Main interactions:
- Electrostatic and coordination interaction between zinc ion and carboxyl groups.
- H-bond and electrostatic interaction between Lys-164A and carboxyl group.



COMPUTATIONAL METHODS

Chart 2
Computational steps performed for each complex

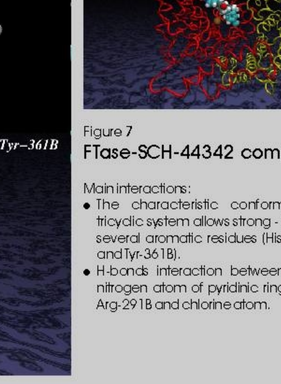


Chart 1
BioDock schematics

BioDock[16,17,18] is a 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 rapid an efficient way. In the present version, both the compound are kept fixed. For each compound, about 10.000.000 complexes have been screened using energetical and sterical criteria implemented in BioDock. Like showed in Chart 1, the 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 in the trajectory file.

DISCUSSION

The Table 1 shows the main interactions and the pIC_{50} for each FTase-inhibitor complex. The Table 2 underlines the aminoacidic residues involved in the ternary complex FTase-HRAS-1-FPP. With the exception of *Lys-164A*, the substrates interact with different residues, because are placed in two different sites (see Figure 1).
In the *(S)-FPOH* complex (see Table 1 and Figure 5) the inhibitor is not coordinated with the zinc ion although is placed in the same site of FPP. The cause of this missing interaction is the unfavourable presence of the oxydril . With the exception of SCH-44342, the other inhibitors coordinate the zinc. The inhibitors table (Table 1) shows that *Arg-291B, His-248B* and *Tyr-361B* are the main aminoacidic residues implicated in the complex stabilization. *BZA-2B* has extra interactions with aminoacidic groups (*Asp-359B* and *His-362B*) that are not related to the formation of all other complexes including the FTase-HRAS-FPP complex.
Thus, these results provide qualitatively acceptable hints for an interpretation of the different behavior of ligands vs. the FTase.

Ligand	pIC_{50}	Zn	Arg-291B	His-248B	Tyr-361E	Tyr-300E	Lys-294E	Lys-164A	Trp-106E	Ser-357B	Arg-202B	Lys-356E	Asp-359B	His-362B
(S)-FPOH[9]	7,82		+	+		+	+							
ZARA[19]	7,92	+			+			+		+				
DESME[20]	4,68	+			+		+				+	+		
FUSID[12]	6,52	+	+	+		+								
BZA-2B[10]	9,07	+	+	+	+								+	+
SCH-44342[11]	6,60		+	+	+	+								

ZARA = Zaragozic acid, DESME = 10'-Desmethoxystreptonigrin, FUSID = Fusidienol

Table 1 - Main interactions and pIC_{50} of FTase-inhibitor complexes.

Substrate	Zn	Arg-291B	His-248B	Tyr-361E	Tyr-300E	Lys-294B	Lys-164A	Trp-106E	Ser-357B
FPP	+	+	+		+	+	+		
H-RAS-1 CVLS	+			+			+	+	+

Table 2 - Main interactions of FTase- HRAS-FPP complex.

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