



Published in final edited form as:

Biochemistry. 2010 November 9; 49(44): 9658–9666. doi:10.1021/bi1008358.

Finding a Needle in the Haystack: Computational Modeling of Mg²⁺ Binding in the Active Site of Protein Farnesyltransferase

Yue Yang, Dhruva K. Chakravorty, and Kenneth M. Merz Jr *

Department of Chemistry and the Quantum Theory Project, 2328 New Physics Building, P.O. Box 118435, University of Florida, Gainesville, Florida, 32611-8435

Kenneth M. Merz: merz@qtp.ufl.edu

Abstract

Studies aimed at elucidating the unknown Mg²⁺ binding site in protein farnesyltransferase (FTase) are reported. FTase catalyzes the transfer of a farnesyl group to a conserved cysteine residue (Cys1p) on a target protein, an important step for proteins in the signal transduction pathways (e.g. Ras). Mg²⁺ ions accelerate the protein farnesylation reaction by up to 700-fold. The exact function of Mg²⁺ in catalysis and the structural characteristics of its binding remain unresolved to date. Molecular Dynamics (MD) simulations addressing the role of magnesium ions in FTase are presented, and relevant octahedral binding motifs for Mg²⁺ in wild type (WT) FTase and Dβ352A mutant are explored. Our simulations suggest that the addition of Mg²⁺ ions causes a conformational changes to occur in the FTase active site, breaking interactions known to keep FPP in its inactive conformation. Two relevant Mg²⁺ ion binding motifs were determined in WT FTase. In the first binding motif, WT1, the Mg²⁺ ion is coordinated to D352β, zinc-bound D297β, two water molecules, and one oxygen atoms from the α- and β-phosphates of farnesyl diphosphate (FPP). The second binding motif, WT2, is identical with the exception of the zinc-bound D297β being replaced by a water molecule in the Mg²⁺ coordination complex. In the Dβ352A mutant Mg²⁺ binding motif, D297β, three water molecules and one oxygen atom from the α- and β-phosphates of FPP complete the octahedral coordination sphere of Mg²⁺. Simulations of WT FTase, in which Mg²⁺ was replaced by water in the active site, re-created the salt bridges and hydrogen bonding patterns around FPP, validating these simulations. In all Mg²⁺ binding motifs, a key hydrogen bond was identified between a magnesium bound water and Cys1p, bridging the two metallic binding sites, and thereby, reducing the equilibrium distance between the reacting atoms of FPP Cys1p. The free energy profiles calculated for these systems provide a qualitative understanding of experimental results. They demonstrate that the two reactive atoms approach each other more readily in the presence of Mg²⁺ in WT FTase and mutant. The flexible WT2 model was found to possess the lowest barrier towards the conformational change, suggesting it is the preferred Mg²⁺ binding motif in WT FTase. In the mutant, the absence of D352β makes the transition towards a conformational change harder. Our calculations find support for the proposal that D352β performs a critical role in Mg²⁺ binding and Mg²⁺ plays an important role in the conformational transition step.

Protein farnesyltransferase (FTase) catalyzes the transfer of a farnesyl group to a cysteine residue (Cys1p) on a CaaX motif (a=aliphatic, X=Met) at or near the C-terminus of a protein substrate(1,2) (see Figure 1). Farnesylation is a critical step in the posttranslational

Corresponding author: Kenneth M. Merz, Jr, Phone: 352-392-6973, Fax: 352-392-8722, merz@qtp.ufl.edu.

Supporting Information Available

The PDB files of WT and MUT FTase systems, along with several other helpful figures. This material is available free of charge online at <http://pubs.acs.org>.

modification of the Ras family of proteins(3–7). Mutations in these proteins are associated with almost 50% of tumors in human-colon-carcinomas and 30% of human cancers. The potential to prevent unmitigated cell growth by inhibiting mutant Ras activity has made the FTase catalyzed farnesylation reaction the subject of numerous studies(2,8–24). Several FTase inhibitors (FTIs) have entered different clinic trial phases(2,25–31). FTase is a zinc metalloenzyme composed of two subunits, with the active site located in a funnel shaped cavity within the β subunit. Computational, kinetic, structural and mutagenesis investigations have revealed the nature of this active site. The two reacting atoms, namely C₁ of the substrate farnesyldiphosphate (FPP) and S γ of Cys1p, are 7 Å (see Figure 2) apart in the crystal structures of the ternary complex (FTase-FPP-peptide substrate). The protein substrate coordinates to the zinc ion through S of Cys1p, forming a tetrahedral zinc complex along with the enzymatic residues D297 β , C299 β and H362 β . The direct coordination of the reactive sulfur atom to the zinc ion, suggests that the zinc ion is involved in the catalytic reaction(32). The exact nature of the reaction pathway has been a source of debate. Recent kinetic isotope effects (KIE) experiments by the Fierke (33) and Distefano groups (20) have indicated that the reaction adopts a S_N2-like associative pathway mechanism, with partial carbocation character (S_N1-like) in the transition state structure.

Mg²⁺ ions have been found to enhance the rate of product formation by ~700-fold without affecting the affinity of either the peptide or FPP(34). The exact function of Mg²⁺ ions has been a subject of recent interest, and is not entirely understood. A magnesium ion has been determined to be bound in the active site, though the exact nature of its binding remains to be resolved by structural analysis. Kinetic and mutagenesis experiments performed by Fierke and coworkers(34), in conjunction with crystallographic studies of FTase with Mn²⁺ ions carried out by Beese and coworkers(2), have provided insights into the characteristics of Mg²⁺ ion binding in the active site. It is widely accepted that bound Mg²⁺ forms an octahedral complex with negatively charged groups. In a proposed model, Mg²⁺ coordinates to a non-bridging phosphate oxygen of α - and β -phosphate in FPP, the carboxylate side chain of D352 β , a carboxylic oxygen of zinc bound D297 β , and a water molecule(34). D352 β has been identified to be crucial for Mg²⁺ ion binding in the active site, with mutations (D β 352A and D β 352K) diminishing the rate enhancing ability of Mg²⁺ ions. It has been proposed that the formation of a D297 β bridged bimetal catalytic site helps reduce the 7 Å distance gap between the two reacting atoms, bringing the enzyme into a pre-organized state for the chemical step. It is also thought that Mg²⁺ ions promote the farnesylation reaction by stabilizing the developing negative charge on the diphosphate (PPI) leaving group, and increasing the carbocation character of C1 in the chemical step.

Computational studies have aided in understanding the FTase catalyzed farnesylation reaction. Cui *et al.* have studied the substrate binding and conformational activation of FTase in the absence of Mg²⁺ ions using MD simulations and free energy sampling methods(17,18). They found that the highly charged binding pocket kept FPP³⁻ locked in an inactive conformation, and the monoprotonated diphosphate group (FPP²⁻) was the only form of FPP that could activate the conformational transition via rotation of the bond between the first two isoprenoid groups. The Ramos group's computational efforts to explain the so-called "distance paradox"(35) agreed with the earlier Merz *et al.* study, that conformational activation is attributed to the isoprenoid-rotation mechanism. More recently, Klein and co-workers investigated the reaction pathway using a quantum mechanical/molecular mechanical (QM/MM) approach(19). Their results suggest that the reaction goes through "an associative pathway with dissociative characteristics", in agreement with experimental observations. This study focused on the chemical step, and did not explore the prior conformational change in the system. However, the system investigated, FTase/FPP³⁻ without Mg²⁺ is a system that is not capable of the necessary conformational change to bring the two reactive centers into contact. Moreover, it is a system that has not been

observed experimentally because FPP³⁻ under these conditions is likely protonated. Crucially, in all of the studies reported to date the role of Mg²⁺ has not been examined leaving a large gap in our understanding of FTase activity under physiological conditions. Nonetheless, previous studies have demonstrated the efficacy of computational methods in exploring FTase activity, and have opened the way to explore the catalytic reaction in the presence of Mg²⁺ ions.

In this paper we present a computational study aimed at locating the Mg²⁺ binding site in FTase. We use MD simulations and free energy calculation methods to elucidate the function of Mg²⁺ ions in the conformational transition step of the reaction. The role of D352 β in binding a magnesium ion in the active site is also investigated in this study. We perform calculations on WT FTase and the D β 352A single mutant with FPP³⁻ and CVIM peptide as substrates, in the presence of an active site bound Mg²⁺ ion. The D β 352A mutation was examined in order to reproduce and provide molecular-level insights into experimental results. We validate our simulations by replacing the active site bound Mg²⁺ ion with a water molecule, and compare the results from this simulation to previously published results. In the absence of crystal structures of FTase with bound Mg²⁺ ion in the active site, we generate starting structures using a methodology successfully utilized earlier by Merz(36) to identify CO₂ binding sites in human carbonic anhydrase II. We start with several “educated” guesses as starting structures for WT and mutant FTase/FPP³⁻/Mg²⁺ complexes based on experimental data. We then identify relevant Mg²⁺ binding motifs and investigate them using MD simulations. We analyze our simulations for hydrogen-bonding patterns, and conformational changes across these motifs. We also calculate the potential of mean force (PMF) for the conformational change in FPP for every motif. The results from our investigations provide new insight into the role of Mg²⁺ ions in helping FPP undergo the conformational transition in the farnesylation reaction, and the associated experimentally observed rate enhancement. We identify a critical hydrogen-bonding pattern in WT FTase, and test the proposal for a D297 β bridged bi-metallic catalytic site. These simulations provide a physical basis for the function of D352 β in FTase, both with and without magnesium ions. Based on the results from our calculations, we propose a novel model for Mg²⁺ binding in the active site of FTase that can be experimentally tested.

The following section of this paper introduces the computational methods used to study the FTase reaction. In this section, we describe methods used to generate and investigate possible Mg²⁺ binding motifs. In Section 3, we present our analyses of the various systems. In the first part of this section, we present the results from modeling mutant and WT FTase with Mg²⁺ ions. The second part of this section analyzes the results from our simulation without bound magnesium in the active site. In the third part of the section, we present the results from our free-energy calculations. Our conclusions from this study are presented in Section 4. We explain the impact of mutations on the enzyme and, finally, we describe our proposed motif for Mg²⁺ binding in the FTase active site.

Methodology

1. Initial Structural Models

The starting structure of WT FTase was generated using the protocol from a previous study of the FTase reaction performed by Merz and coworkers(17). In brief, the reactive form of FTase/FPP complexed with the substrate peptide (acetyl-CVIM) was obtained by superimposing two crystal structures (PDB codes: 1QBQ and 1FT2). All crystal water molecules were retained. The AMBER force field *ff99sb* by Hornak *et al.* was applied to the system(37). Charged amino acid chains were modeled in their normal protonation states at physiological pH, and all histidine residues were treated as neutral. At physiological pH, Cys1p has been found to be deprotonated, and was modeled as a thiolate(38). It was

coordinated to the zinc ion along with D297 β , C299 β and H362 β from the protein. The zinc parameters previously derived by Cui *et al.* were used for all zinc complexes reported in this paper(39). The VDW parameters, $\epsilon=1.10$ and $r_0=0.0125$ (40), were used for zinc. The AMBER 10 suite of programs(41) was chosen for all molecular mechanics and MD simulations. FPP was represented in its entirely deprotonated state, FPP³⁻, with 3 units of negative charge. The protonation of PPI leads to a loss of Mg²⁺ binding affinity(42), making FPP²⁻ unimportant for the present study. The parameters used to model FPP were obtained from the generic AMBER force field (GAFF)(43) for small organic molecules. In addition, torsion parameters for the farnesyl group developed by Merz and coworkers(17), and diphosphate parameters developed by Carson and coworkers were used(44). The restrained electrostatic potential (RESP) charges for FPP³⁻ were derived using the prescribed procedure for the AMBER force field(45,46). In accordance with the procedure, a geometry optimization of FPP was performed using density functional theory at the B3LYP/6-31G* level with Gaussian 03(47). The electrostatic potential derived atomic point charges were then calculated at the HF/6-31G* level. Finally, the atomic charges were derived following a two-stage RESP fitting procedure.

2. Magnesium in FTase Modeling and MD

We examined the FPP binding pocket for aspartic acid residues proximal to PPI. D196 α , D297 β and D352 β were identified as potential candidates for Mg²⁺ ion binding. Based on experimental and theoretical evidence, the sequence conserved D297 β and D352 β residues were recognized as the most possible Mg²⁺ binding ligands. We created “guess” starting structures for our simulations by positioning a magnesium ion at different sites within the binding pocket (see Figure 3). In these structures, the Mg²⁺ ion was positioned close to negatively charged PPI and one of the three candidate aspartic acid residues. Neighboring crystal water molecules completed the octahedral coordination of the Mg²⁺ ion. A total of 10 guess structures were produced. These systems are referred to as FTase/FPP³⁻/Mg²⁺ in the remainder of this text. The AMBER default VDW parameters were used for magnesium ($\epsilon=0.7926$, $r_0=0.8947$)(48). Attempts to find such binding structures using local enhanced sampling (LES) were unsuccessful. This was likely caused by the significant charge on the Mg²⁺ ion.

Each FTase/FPP³⁻/Mg²⁺ system was solvated in a periodically replicated truncated octahedral water box with each side of the box at least 10Å away from the closest solute atom. The three-site constraint TIP3P water model was employed(49), and counter ions were added to neutralize the net negative charge of the system. Long range electrostatic interactions were calculated by the particle mesh Ewald (PME)(50) method. An 8Å cutoff was used for the nonbonded interactions in real-space, along with a 1Å reciprocal space grid spacing. The SHAKE(51) algorithm was applied to constraint the length of covalent bonds in which hydrogen atoms were involved, and the translational movement of the system was removed. 5000 steps of steepest descent minimization was carried out with a weak harmonic positional restraint applied to the enzyme, substrate peptide and zinc complex, followed by a 5000-step conjugate gradient minimization. Next, the system was heated to 300K over 100ps of MD with 1fs time steps for a canonical ensemble (NVT). The temperature of the system was maintained using Langevin dynamics with a 1 ps⁻¹ collision frequency. All restraints were released prior to data collection. A 6ns MD trajectory for an isobaric, isothermal ensemble (NPT) at 1atm was propagated with a 2fs timestep. Snapshots were saved every picosecond, and the resulting trajectories from the last 4ns of the simulation were examined to validate reasonable Mg²⁺ binding motifs. The D297 β bridged Zn-Mg site binding motif featuring a bimetallic catalytic site, was artificially constructed by using an NMR restraint to force D297 β to displace one of the Mg²⁺ bound water molecules in a D352 β bound model. This motif was investigated using the scheme described above.

3. D β 352A Mutation

We prepared the D β 352A mutant form of FTase from the final snapshot of a successful D352 β binding Mg $^{2+}$ binding motif. All counter ions, and water molecules located more than 6Å away from the substrate FPP $^{3-}$ or Mg $^{2+}$ were removed. D352 β was modified as an Alanine, leaving D297 β as the only neighboring aspartic acid residue. This system is referred to as MUT in this paper. The resulting MUT system was immersed in a truncated octahedral box and neutralized with counter-ions. The system was minimized to remove strain from the residue modification. The MUT system was then simulated using the same procedures described for WT FTase.

4. Mg $^{2+}$ /H $_2$ O Switch Simulation

We removed the bound Mg $^{2+}$ ion from the active site of a successful FTase/FPP $^{3-}$ /Mg $^{2+}$ simulation to further validate our Mg $^{2+}$ bonding model. A straightforward method was adopted wherein the coordinates and velocity information of the bound Mg $^{2+}$ ion and the oxygen atom of a bulk water molecule, denoted H $_2$ O $_{\text{swap}}$, were exchanged. The coordinates of the two H $_2$ O $_{\text{swap}}$ hydrogen atoms were modified suitably, while their velocities were preserved. The remaining parts of the system were untouched. The resulting system is referred to as SWAP in the remainder of this paper. The SWAP system was studied with the same MD procedure used for WT FTase with active site bound Mg $^{2+}$ ion. 4 ns of equilibration followed by 4 ns of NPT MD simulations were performed for this system.

5. Potential of Mean Force Studies

We calculated the potential of mean force (PMF) as a function of the distance between the two reacting centers, namely C $_1$ of FPP and S γ of the target cysteine peptide for the various systems investigated in this study. In these calculations, the reactive atoms were “pulled” from the crystal structure distance of 8.0Å to 4.5Å. A PMF termination distance of 4.5Å was chosen based on the sum of the van der Waals (VDW) radii of sulfur and oxygen in the AMBER *ff99SB* force field since sampling below this distance would likely yield spurious results. We obtained the initial structures for the umbrella sampling(52) calculations from a MD scanning procedure driven by a large harmonic force. The reaction coordinate (RC) was divided into 15 windows spaced 0.25Å apart. A harmonic potential of 20kcal/(mol•Å 2) was applied to each window during a 2ns simulation with a 2fs time step. Data was collected every 5 steps after 1ns of equilibration; therefore, 10,000 snapshots were saved for every window and at least 100,000 points were stored for the following analysis. Longer periods of sampling were performed in the regions near the intermediate or transition barrier to improve the overlap between adjacent windows. An increased force constant of 100kcal/(mol•Å 2) was used with 29 biasing potentials for systems unable to sample the entire span of the RC. Grossfield’s weighted histogram analysis method (WHAM) (53) program was used to reconstruct the unbiased distribution of the reaction coordinate and then extracted the free energy profile.

Results and Discussions

1. FTase/FPP $^{3-}$ /Mg $^{2+}$ Modeling and Simulation

By manually placing Mg $^{2+}$ into the FTase/FPP $^{3-}$ ternary complex at different locations, a series of Mg $^{2+}$ ion binding motifs in the active site of WT FTase were generated. As described above, these binding motifs were then validated using MD simulations. In all, three possible Mg $^{2+}$ binding models were detected for WT FTase, and are displayed in Figure 3. All three candidate amino acids, D196 α , D352 β and D297 β were found involved in one or more of these schemes. A D297 β bridged bimetallic catalytic site was observed in the first motif, in which the Mg $^{2+}$ ion coordinated to two water molecules, an oxygen atom

from both phosphate groups of FPP, a single carboxylic oxygen of D352 β and a single carboxylic oxygen of D297 β . In the second Mg²⁺ binding motif, the octahedral Mg²⁺ binding site involved two oxygen atoms from the FPP substrate with both the α - and β -phosphate groups contributing one oxygen atom each, a carboxylate oxygen of D352 β and three water molecules. In the third motif, Mg²⁺ was octahedrally coordinated to an oxygen atom of the α -diphosphate of FPP, a carboxylate oxygen of D196 α and four water molecules. These binding schemes, will be denoted as WT1 (D352 β + D297 β), WT2 (D352 β), and WT3 (D196 α), respectively in the remainder of this article.

The Mg²⁺ ion binding in the active site was found to be reasonable in all three cases via visual inspection. The bound Mg²⁺ ion in all motifs displayed octahedral coordination. Each motif has at least one aspartate side chain participating, and all of them were observed to have direct contacts with the diphosphate oxygens. We found these binding motifs to be stable over 6ns of MD simulations. The distance between the reacting atoms (the distance between S γ and C $_1$ = d_{RC}) for the three schemes were calculated from their MD trajectories and this term is used through out the manuscript in this context. For the WT1 and WT2 binding motifs, the calculated average values of d_{RC} indicated that the distance between the reacting atoms reduced to below 6.5 \AA , as compared to value of 7.2 \AA in the FTase/FPP²⁻ case in the absence of magnesium ions. These results suggest that WT1 and WT2 are structurally more favored than FTase/FPP²⁻ to move the system from the resting state to a reaction ready form with a d_{RC} < 4.0 \AA . In contrast to WT1 and WT2, the computed d_{RC} values for the WT3 binding motif showed the average distance between the reacting atoms to have significantly increased to ~10 \AA (~50% increase in length). The increased distance found in the WT3 binding motif makes it less likely to transition to the chemical step. In this motif, moreover, the involvement of only the α -phosphate oxygen is inconsistent with the model proposed by Fierke and coworkers(34,42). Although the possibility of D196 α being involved cannot be entirely ruled out, for the purposes of this study, we chose to pursue the more probable WT1 and WT2 binding motifs.

D297 β and D352 β are highly conserved residues, which implies an important role for them in FTase chemistry. Mutagenesis experiments have found D352 β to be essential for the 700-fold increase in the rate of product formation associated with the presence of Mg²⁺(34). The results from our simulations support the assumption that the Mg²⁺ in the active site makes a contribution towards the conformational transition that closes the 7 \AA gap between the reacting atoms and stabilizes the developing negative charge on PPi in the chemical step. The involvement of both α - and β -phosphate oxygen atoms in Mg²⁺ ion coordination is consistent with the experimentally proposed model. We regards these motifs featuring the Mg²⁺-D352 β interaction as our best estimate of the Mg²⁺ binding motifs in the WT FTase/FPP³⁻/Mg²⁺ system.

We observed similarities in the active sites of WT FTase across both motifs. The active site conformation and interaction patterns from simulations of WT1 and WT2 are shown in Figure 3. A key hydrogen bond was identified between Cys1p and a magnesium ion bound water molecule. The tetrahedral coordination of the zinc ion was well preserved in both systems. The bound Mg²⁺ ion was found to form strong electrostatic interactions with PPi, disrupting the previously existing R291 β -FPP³⁻ and K294 β -FPP³⁻ salt bridge interactions. Cui and Merz calculated a more than 5kcal/(mol $\cdot\text{\AA}$) free energy penalty for breaking these salt bridge interactions, making the conformational transition of FTase/FPP³⁻ energetically unfavorable in the absence of Mg²⁺ ions(18). As a result of D352 β approaching FPP³⁻ to form the Mg²⁺ binding site, its adjacent residue, positively charged K353 β , also moves towards PPi and forms a hydrogen bond to one of the β -phosphate oxygen atoms, further stabilizing the leaving group during the chemical step.

Y300 β was observed to be important to the farnesylation reaction based on mutagenesis experiments, but was not found in our earlier MD simulations to form clear interactions with the resting state of FTase/FPP³⁻, while acting as a hydrogen bond donor to PPi in the FTase/FPP²⁻ study. However, in this study, it moves away from the PPi and provides a hydrogen bond to the acetyl capping group, which can be viewed as further stabilizing the peptide substrate. On the other hand, another positively charged residue close to FPP³⁻, K164 α , still forms a salt bridge interaction with the α -phosphate oxygen. It is noteworthy that in the previous FTase/FPP²⁻ PMF study, the roles of K164 α and Y300 β were switched, with the lysine residue forming a hydrogen bond with the capping acetyl group, while the tyrosine residue formed a hydrogen bond with PPi. While their roles appear to exchange depending on the active site environment and position along the reaction coordinate, ultimately, these two groups help stabilize the peptide and the phosphate during the chemical step.

The two binding motifs differ in the function of zinc bound D297 β . The WT1 binding motif is a D297 β bridged bimetallic binding site, broadly similar to the model proposed by Fierke and co-workers(34). The two metal ions are 3.3 \AA apart, and the calculated equilibrium distance between the reacting atoms was 5.8 \AA , suggesting this binding scheme might be energetically favored over WT FTase/FPP^{2-/3-} in the absence of Mg²⁺ ions. MD simulations of this motif found the distance between the reactive atoms to fluctuate over a small range. This suggests that the D297 β bridge possibly constrains the system, making the active site less mobile. Such a constrained environment would make it harder for the reactive atoms to approach one another. The WT2 binding motif does not contain the D297 β bridged bimetallic catalytic site. A water molecule replaces D297 β in the magnesium coordination complex, allowing the positively charged zinc and magnesium ions to be separated by 5.1 \AA . The reactive atom distances from MD simulations of this model were found to fluctuate around 6.1 \AA . This suggests that the hydrogen bond observed between Cys1p and a Mg²⁺ ion bound water molecule functions as a substitute bridge between the two metal binding sites, and helps reduce the gap between the reacting partners. The larger fluctuation in the WT2 d_{RC} suggests a more flexible binding site, which will allow the reactive atoms to reach a pre-organized state essential for the chemical step.

In contrast to the model proposed by Fierke and co-workers, D352 β does not donate both oxygen atoms from its carboxylate group to Mg²⁺ in either binding motif for WT FTase. Instead only one oxygen atom participates. In order to test the possibility of the Mg²⁺-D352 β double interaction scheme, tests were conducted that forced Mg²⁺ to establish a second interaction to both D352 β carboxylate oxygen atoms by applying a harmonic force between the two atoms; however, a stable structure was not observed.

2. D β 352A Mutation and Mg²⁺/H₂O Swap Simulation

In the experimental mutagenesis study of D β 352A in the presence of Mg²⁺ ions, a ~30-fold difference in the product formation rate constant was observed. With Mg²⁺ losing one of its favored coordinating ligands, D352 β , another candidate aspartic acid, D297 β , can be assumed to replace it in the magnesium coordination shell. A second series of MD simulations were carried out to study the impact of this mutation and account for the nearly 2kcal/mol increase in the free energy barrier. As previously described, the WT2 motif was used as the starting structure for this mutation. A new Mg²⁺ binding scheme was observed and was verified by MD simulations. The new binding motif was found similar to the previous case of WT1. In this new motif, a water molecule replaces D352 β to complete the Mg²⁺ coordination sphere (see Figure 3) and D297 β serves as a bridge residue connecting the two metal binding sites together into what appears as a bimetallic site. The two metal cations are separated by 3.4 \AA , similar to the binding motif observed in WT1, but considerably shorter than before the mutation (5.1 \AA for WT2). It is interesting that Mg²⁺ binds to the sequence conserved D297 β in the absence of D352 β .

Inside the catalytic pocket, several key hydrogen bonds are well preserved, including those between K164 α and the α -phosphate of FPP, K353 β and β -phosphate, and Y300 β and the capping acetyl group. To further stabilize PPi, several water molecules surrounding it were found to form hydrogen bonds with the free β -phosphate oxygen. H248 β was observed to form a hydrogen bond with Y300 β instead of PPi. Interestingly, in addition to the D297 β bridged bimetallic site, two Mg²⁺ bound water molecules are now found to interact with the sulfur atom of the peptide *via* a hydrogen bond, which helps shortens d_{RC} . The equilibrium value of d_{RC} , however, increased from 6.1 in WT2 to 6.5Å, but was still considerably shorter than that of the resting state of FTase/FPP^{2-/3-} without Mg²⁺. Based on the different equilibrium values of d_{RC} in the different FTase/FPP systems, the difficulty of undergoing the conformational step prior to the chemical steps was predicted to follow the order of: FTase/FPP²⁻ > MUT > WT1/WT2. The real trend, however, cannot be determined without carrying out a series of PMF studies for each of the systems using hybrid QM/MM methods covering the entire length of the RC from ~8.0Å to the covalent bonding distance of ~1.8Å. Such a study is currently underway in our laboratory.

A third set of MD simulations was carried out on the SWAP system. In this system, the active site bound Mg²⁺ ion was exchanged with a bulk solvent water molecule in order to study the impact of losing Mg²⁺ in the active site of FTase. The ideal result would be to relax to the FTase/FPP³⁻ starting structure in the absence of Mg²⁺. As expected, a binding pocket similar to FTase/FPP³⁻ was obtained (see Figure 3). The key interactions observed in the resting state of FTase/FPP³⁻ were largely restored, including the hydrogen bond between H248 β and the diphosphate, the salt bridge between K294 β and the β -phosphate oxygen, and the double interaction between R291 β and an α -phosphate oxygen and the diphosphate bridging oxygen. These interactions have been shown to “lock” FPP³⁻ in its conformational state, and prevent it from undergoing a transition in its conformation prior to the chemical step. At the same time, D352 β was found to move away from the FPP³⁻ substrate. In the absence of Mg²⁺, the Cys1p residue was found to move away, leaving an equilibrium d_{RC} of ~7.4Å, similar to the global minimum found for FTase/FPP³⁻ by Cui and Merz(18).

3. Potential of Mean Force (PMF) Studies

Computing the free energy has not been a straightforward task in molecular modeling and simulations. The main difficulty comes from the inability of general MD simulations to overcome free energy barriers and adequately sample the phase space in regions such as transition or intermediate states. The implementation of special techniques, for instance, umbrella sampling, allows for the study of the free energy surface along a specified RC (D_{C1-S_Y}). 15 simulations were carried out with anchor points placed between 8.0 and 4.5Å for WT2, MUT and SWAP systems. To efficiently sample the RC for WT1, a stronger bias was employed and trajectories were propagated at 29 anchor points.

We found that the inclusion of Mg²⁺ into the active site altered the free energy profile. For WT1, the global minimum shifted from 7.4Å in FTase/FPP³⁻ to 5.8Å, decreasing the distance between the two reacting atoms by 1.8Å. The free energy profile, however, suggests that this might not be the correct binding motif for Mg²⁺ in the active form of FTase. With steep increases in free energy found on both side of this equilibrium point, the system appears locked into this minimum position, with the reactive atoms unlikely to get closer. This in association with small fluctuations observed in the distance between the two reactive atoms during MD simulations, lead to the conclusion that the bridged bimetallic Zn²⁺-D297 β -Mg²⁺ active site in WT1 is very rigid. In WT2, we found the global minimum shifted to 6.1Å, corresponding to a larger distance (by 0.3Å) between the reacting atoms compared to WT1. Also, a second minimum corresponding to an intermediate state was found at around 5.0Å, in a location similar to that for FTase/FPP²⁻. The intermediate itself has a free energy approximately 0.5kcal/mol higher than that of the resting state, and the free

energy barrier for the conformational transition is about 0.8kcal/mol, slightly lower than that observed for the FTase/FPP²⁻ system. With such a low transition barrier and a flat energy profile, the approach of the two reacting centers is facilitated. This is supported by d_{RC} distributions (see SI) extracted from the last 4ns of the MD simulation; another observation supported by the d_{RC} distribution map is the existence of a second minimum under 5.5Å in addition to the global minimum. Moreover, the free energy penalty for further shortening d_{RC} beyond the intermediate state is significantly reduced from ~1.7kcal/mol (in FTase/FPP²⁻) to ~1.0kcal/mol in WT2, meaning the two reactive atoms can more readily approach each other in this relatively flexible active site model. However, due to the possible strong VDW clashes at distances close to 4.5Å, this finding needs to be further explored by QM/MM PMF's for distances shorter than 4.5Å.

The PMF calculated for the D352A mutant found a global minimum corresponding to a reactive atom distance of ~6.6Å, 0.5Å longer than that of WT2. From the MD simulation, the equilibrium d_{RC} distance was found to oscillate around 6.6Å. An intermediate state was not observed, and a much larger free energy penalty, up to ~6kcal/mol, was observed close to 4.5Å. Experimental measurements suggested an approximately 30-fold difference in the rate constant of product formation meaning a 2kcal/mol higher free energy barrier than FTase/FPP³⁻/Mg²⁺. Assuming that the difference can be entirely ascribed to the conformational step, our observation of a 5kcal/mol energy difference (6kcal/mol - ~1kcal/mol) is too large. The difference can be made up in the chemistry steps, but this has yet to be determined. The free energy profile also shows that all regions between 5.5Å and the resting state are accessible with only a ~1kcal/mol free energy difference. Regardless, we find that the Dβ352A has a significant effect on the conformational step and it is left to be determined how it affects subsequent chemical steps.

Finally, a fourth PMF study was carried out on the SWAP system that aimed to reproduce the free energy profile obtained from the wild type FTase/FPP³⁻ system in the absence of Mg²⁺. As expected, a similar free energy profile to the case of FTase/FPP³⁻ without Mg²⁺ was obtained. Comparing our plot (see Figure 4) to that published by Cui and Merz (denoted as FTase/FPP³⁻/CVIM), excellent agreement is found: both the 7.5Å global minimum and the free energy jump beyond that point are well reproduced. Overall, the agreement is quite good with only minor differences observed. Hence, the present study recapitulates our earlier effort and further confirms the reasonableness of our active site model for Mg²⁺ bound to FTase.

Conclusion

We modeled and validated several FTase active site magnesium binding schemes using MD simulations and classical PMF studies with umbrella sampling. Three possible active site models were observed for wild type FTase/FPP³⁻/Mg²⁺ (see Figure 3), but only one was consistent with all available experimental information. In this motif, WT2, Mg²⁺ adopts an octahedral coordination formed with an oxygen atom from both α - and β -diphosphate, a carboxylate oxygen from D352 β and three water molecules (see Figure 3). The involvement of zinc ion bound D297 β , creating a bridged bimetallic catalytic site is not found to be a reasonable option for Mg²⁺ binding in FTase/FPP³⁻/Mg²⁺. An interesting hydrogen bond formed between a magnesium ion bound water molecule and Cys1p was detected, which contributes to the reduction of d_{RC} . Dβ352A/FPP³⁻/Mg²⁺ was also simulated and a single active site model emerged for this system as well (see Figure 3). Free energy calculations show that Mg²⁺ ions allow the reacting atoms to approach each other more readily. Our simulations show that WT FTase/FPP³⁻/Mg²⁺ and the MUT systems both have resting states (6.1Å and 6.5Å, respectively) with a significantly shortened equilibrium d_{RC} relative to FTase/FPP³⁻ (7.4Å) but they experience different free energy profiles beyond this point.

With a free energy difference of less than 1kcal/mol from the resting state to the lower end of our PMF study at 4.5Å, the whole RC range is accessible for the WT FTase/FPP³⁻/Mg²⁺ system. This ready accessibility, over the entire RC range supports the isoprene-rotation mechanism for the conformational transition. On the other hand, a nearly 6kcal/mol free energy penalty from the resting state to 4.5Å makes the approach of FPP³⁻ to the target cysteine residue much more difficult for the mutant.

Introducing Mg²⁺ into the FTase/FPP³⁻ active site is probably the only way in which FPP³⁻ can approach the peptide substrate bound to the zinc ion. The highly charged binding pocket has been shown to freeze FPP in its conformation in FTase/FPP³⁻ in the absence of Mg²⁺, preventing the transition required before the chemical step. With Mg²⁺ included, the resting state is significantly perturbed and the free energy barrier for the conformational transition is dramatically reduced. Binding of Mg²⁺ in FTase/FPP³⁻ pulls the diphosphate away from the positive charged pocket observed in the FTase active site, especially R291β and K294β. Mg²⁺ compensates for the lost salt bridge interactions by coordinating to both the α- and β-diphosphate oxygen atoms. K164α is 'locked' into an interaction with an oxygen from the α-phosphate even with Mg²⁺ binding to FTase, regardless whether D352β was mutated or not, in accordance with the experimental evidence that this interaction is important for stabilizing the diphosphate leaving group during the chemical step. When Mg²⁺ was swapped out of the active site with a bulk water molecule we were able observe the relaxation back to the resting state for FTase/FPP³⁻ in the absence of Mg²⁺ with a d_{RC} minimum of 7.5Å. Moreover, key hydrogen bonds between the diphosphate and residues like H248β, R291β and K294β were reformed, implying that Mg²⁺ is indeed the sole reason for the conformational transition.

Through the use of classical simulations we have identified the elusive Mg²⁺ binding state in FTase/FPP³⁻, thereby, setting the stage for further experimental and theoretical studies aimed at further understanding the catalytic mechanism of this fascinating and important enzyme.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors want to thank Dr. Guanglei Cui and Dr. Bing Wang for numerous helpful discussions. We thank the NIH (GM044974) for funding this research. We also acknowledge the University of Florida High-Performance Computing Center for providing computational resources and support that have contributed to the research results reported within this paper. URL: <http://hpc.ufl.edu>.

Abbreviations

FTase	farnesyltransferase
WT	wild type
FPP	farnesyl diphosphate
FTI	farnesyltransferase inhibitor
KIE	kinetic isotope effect
QM/MM	quantum mechanical/molecular mechanical
MD	molecular dynamics

GAFF	General AMBER force field
RESP	restrained electrostatic potential
LES	local enhanced sampling
PME	particle mesh ewald
PMF	potential of mean force
RC	reaction coordinate
VDW	van der Waals
WHAM	weighted histogram analysis method

References

- Zhang FL, Casey PJ. Protein prenylation: Molecular mechanisms and functional consequences. *Ann Rev Biochem.* 1996; 65:241–269. [PubMed: 8811180]
- Long SB, Casey PJ, Beese LS. Reaction path of protein farnesyltransferase at atomic resolution. *Nature.* 2002; 419:645–650. [PubMed: 12374986]
- Roskoski R. Protein prenylation: A pivotal posttranslational process. *Biochem Bioph Res Co.* 2003; 303:1–7.
- Adjei AA. Blocking oncogenic ras signaling for cancer therapy. *J Natl Cancer Inst.* 2001; 93:1062–1074. [PubMed: 11459867]
- Resh MD. Regulation of cellular signalling by fatty acid acylation and prenylation of signal transduction proteins. *Cell Signal.* 1996; 8:403–412. [PubMed: 8958442]
- Seabra MC. Membrane association and targeting of prenylated ras-like gtpases. *Cell Signal.* 1998; 10:167–172. [PubMed: 9607139]
- Sinensky M. Recent advances in the study of prenylated proteins. *Bba-Mol Cell Biol L.* 2000; 1484:93–106.
- Wu Z, Demma M, Strickland CL, Radisky ES, Poulter CD, Le HV, Windsor WT. Farnesyl protein transferase: Identification of k164 alpha and y300 beta as catalytic residues by mutagenesis and kinetic studies. *Biochemistry.* 1999; 38:11239–11249. [PubMed: 10471273]
- Hightower KE, Casey PJ, Fierke CA. Farnesylation of nonpeptidic thiol compounds by protein farnesyltransferase. *Biochemistry.* 2001; 40:1002–1010. [PubMed: 11170422]
- Pickett JS, Bowers KE, Hartman HL, Fu HW, Embry AC, Casey PJ, Fierke CA. Kinetic studies of protein farnesyltransferase mutants establish active substrate conformation. *Biochemistry.* 2003; 42:9741–9748. [PubMed: 12911316]
- Dunten P, Kammlott U, Crowther R, Weber D, Palermo R, Birktoft J. Protein farnesyltransferase: Structure and implications for substrate binding. *Biochemistry.* 1998; 37:7907–7912. [PubMed: 9609683]
- Long SB, Casey PJ, Beese LS. Cocystal structure of protein farnesyltransferase complexed with a farnesyl diphosphate substrate. *Biochemistry.* 1998; 37:9612–9618. [PubMed: 9657673]
- Long SB, Casey PJ, Beese LS. The basis for k-ras4b binding specificity to protein farnesyltransferase revealed by 2 angstrom resolution ternary complex structures. *Structure.* 2000; 8:209–222. [PubMed: 10673434]
- Long SB, Hancock PJ, Kral AM, Hellenga HW, Beese LS. The crystal structure of human protein farnesyltransferase reveals the basis for inhibition by caax tetrapeptides and their mimetics. *Proc Natl Acad Sci USA.* 2001; 98:12948–12953. [PubMed: 11687658]
- Park HW, Boduluri SR, Moomaw JF, Casey PJ, Beese LS. Crystal structure of protein farnesyltransferase at 2.25 angstrom resolution. *Science.* 1997; 275:1800–1804. [PubMed: 9065406]

16. Strickland CL, Windsor WT, Syto R, Wang L, Bond R, Wu Z, Schwartz J, Le HV, Beese LS, Weber PC. Crystal structure of farnesyl protein transferase complexed with a caax peptide and farnesyl diphosphate analogue. *Biochemistry*. 1998; 37:16601–16611. [PubMed: 9843427]
17. Cui G, Wang B, Merz KM Jr. Computational studies of the farnesyltransferase ternary complex part i: Substrate binding. *Biochemistry*. 2005; 44:16513–16523. [PubMed: 16342942]
18. Cui G, Merz KM Jr. Computational studies of the farnesyltransferase ternary complex part ii: The conformational activation of farnesyldiphosphate. *Biochemistry*. 2007; 46:12375–12381. [PubMed: 17918965]
19. Ho M-H, Vivo MD, Peraro MD, Klein ML. Unraveling the catalytic pathway of metalloenzyme farnesyltransferase through qm/mm computation. *J Chem Theory Comput*. 2009; 5:1657–1666.
20. Lenevich S, Xu JH, Hosokawa A, Cramer CJ, Distefano MD. Transition state analysis of model and enzymatic prenylation reactions. *J Am Chem Soc*. 2007; 129:5796. [PubMed: 17439118]
21. Sousa SF, Fernandes PA, Ramos MJ. Molecular dynamics simulations on the critical states of the farnesyltransferase enzyme. *Bioorgan Med Chem*. 2009; 17:3369–3378.
22. Houglund JL, Lamphear CL, Scott SA, Gibbs RA, Fierke CA. Context-dependent substrate recognition by protein farnesyltransferase. *Biochemistry*. 2009; 48:1691–1701. [PubMed: 19199818]
23. Houglund JL, Hicks KA, Hartman HL, Kelly RA, Watt TJ, Fierke CA. Identification of novel peptide substrates for protein farnesyltransferase reveals two substrate classes with distinct sequence selectivities. *J Mol Biol*. 2010; 395:176–190. [PubMed: 19878682]
24. Bowers KE, Fierke CA. Positively charged side chains in protein farnesyltransferase enhance catalysis by stabilizing the formation of the diphosphate leaving group. *Biochemistry*. 2004; 43:5256–5265. [PubMed: 15122891]
25. Taveras AG, Kirschmeier P, Baum CM. Sch-66336 (sarasar (r)) and other benzocycloheptapyridyl farnesyl protein transferase inhibitors: Discovery, biology and clinical observations. *Curr Top Med Chem*. 2003; 3:1103–1114. [PubMed: 12769711]
26. Doll RJ, Kirschmeier P, Bishop WR. Farnesyltransferase inhibitors as anticancer agents: Critical crossroads. *Curr Opin Drug Disc*. 2004; 7:478–486.
27. Huang CY, Rokosz L. Farnesyltransferase inhibitors: Recent advances. *Expert Opin Ther Pat*. 2004; 14:175–186.
28. Khuri FR, Glisson BS, Kim ES, Statkevich P, Thall PF, Meyers ML, Herbst RS, Munden RF, Tendler C, Zhu YL, Bangert S, Thompson E, Lu C, Wang XM, Shin DM, Kies MS, Papadimitrakopoulou V, Fossella FV, Kirschmeier P, Bishop WR, Hong WK. Phase i study of the farnesyltransferase inhibitor lonafarnib with paclitaxel in solid tumors. *Clin Cancer Res*. 2004; 10:2968–2976. [PubMed: 15131032]
29. Rao S, Cunningham D, de Gramont A, Scheithauer W, Smakal M, Humblet Y, Kourteva G, Iveson T, Andre T, Dostalova J, Illes A, Belly R, Perez-Ruixo JJ, Park YC, Palmer PA. Phase iii double-blind placebo-controlled study of farnesyl transferase inhibitor r115777 in patients with refractory advanced colorectal cancer. *J Clin Oncol*. 2004; 22:3950–3957. [PubMed: 15459217]
30. Lane KT, Beese LS. Thematic review series: Lipid posttranslational modifications. Structural biology of protein farnesyltransferase and geranylgeranyltransferase type i. *J Lipid Res*. 2006; 47:681–699. [PubMed: 16477080]
31. Zhang YH, Cao R, Yin F, Hudock MP, Guo RT, Krysiak K, Mukherjee S, Gao YG, Robinson H, Song Y, No JH, Bergan K, Leon A, Cass L, Goddard A, Chang TK, Lin FY, Van Beek E, Papapoulos S, Wang AHJ, Kubo T, Ochi M, Mukkamala D, Oldfield E. Lipophilic bisphosphonates as dual farnesyl/geranylgeranyl diphosphate synthase inhibitors: An x-ray and nmr investigation. *J Am Chem Soc*. 2009; 131:5153–5162. [PubMed: 19309137]
32. Huang CC, Hightower KE, Fierke CA. Mechanistic studies of rat protein farnesyltransferase indicate an associative transition state. *Biochemistry*. 2000; 39:2593–2602. [PubMed: 10704208]
33. Pais JE, Bowers KE, Fierke CA. Measurement of the alpha-secondary kinetic isotope effect for the reaction catalyzed by mammalian protein farnesyltransferase. *J Am Chem Soc*. 2006; 128:15086–15087. [PubMed: 17117849]

34. Pickett JS, Bowers KE, Fierke CA. Mutagenesis studies of protein farnesyltransferase implicate aspartate beta 352 as a magnesium ligand. *J Biol Chem.* 2003; 278:51243–51250. [PubMed: 14532266]
35. Sousa SF, Fernandes PA, Ramos MJ. Theoretical studies on farnesyltransferase: The distances paradox explained. *Proteins-Structure Function and Bioinformatics.* 2007; 66:205–218.
36. Merz KM Jr. Co2 binding to human carbonic anhydrase-ii. *J Am Chem Soc.* 1991; 113:406–411.
37. Viktor Hornak RA, Okur Asim, Strockbine Bentley, Roitberg Adrian, Simmerling Carlos. Comparison of multiple amber force fields and development of improved protein backbone parameters. *Proteins: Struct, Funct, Bioinf.* 2006; 65:712–725.
38. Hightower KE, Huang CC, Casey PJ, Fierke CA. H-ras peptide and protein substrates bind protein farnesyltransferase as an ionized thiolate. *Biochemistry.* 1998; 37:15555–15562. [PubMed: 9799520]
39. Cui G, Wang B, Merz KM Jr. Computational studies of the farnesyltransferase ternary complex part i: Substrate binding. *Biochemistry.* 2005; 44:16513. [PubMed: 16342942]
40. Merz KM Jr, Murcko MA, Kollman PA. Inhibition of carbonic-anhydrase. *J Am Chem Soc.* 1991; 113:4484–4490.
41. Case, DA.; Darden, TA.; Cheatham, TE., III; Simmerling, CL.; Wang, J.; Duke, RE.; Luo, R.; Crowley, M.; Walker, RC.; Zhang, W.; Merz, KM., Jr; Wang, B.; Hayik, S.; Roitberg, A.; Seabra, G.; Kolossváry, I.; Wong, KF.; Paesani, F.; Vanicek, J.; Wu, X.; Brozell, SR.; Steinbrecher, T.; Gohlke, H.; Yang, L.; Tan, C.; Mongan, J.; Hornak, V.; Cui, G.; Mathews, DH.; Seetin, MG.; Sagui, C.; Babin, V.; Kollman, PA. Amber. Vol. 10. University of California; San Francisco: 2008.
42. Saderholm MJ, Hightower KE, Fierke CA. Role of metals in the reaction catalyzed by protein farnesyltransferase. *Biochemistry.* 2000; 39:12398–12405. [PubMed: 11015220]
43. Wang JM, Wolf RM, Caldwell JW, Kollman PA, Case DA. Development and testing of a general amber force field. *J Comput Chem.* 2004; 25:1157–1174. [PubMed: 15116359]
44. Meagher KL, Redman LT, Carlson HA. Development of polyphosphate parameters for use with the amber force field. *J Comput Chem.* 2003; 24:1016–1025. [PubMed: 12759902]
45. Cieplak P, Cornell WD, Bayly C, Kollman PA. Application of the multimolecule and multiconformational resp methodology to biopolymers: Charge derivation for DNA, rna, and proteins. *J Comput Chem.* 1995; 16:1357–1377.
46. Bayly CI, Cieplak P, Cornell W, Kollman PA. A well-behaved electrostatic potential based method using charge restraints for deriving atomic charges: The resp model. *Journal of Physical Chemistry.* 1993; 97:10269–10280.
47. Frisch, MJT.; GW; Schlegel, HB.; Scuseria, GE.; Robb, MA.; Cheeseman, JR.; Montgomery, JA., Jr; Vreven, T.; Kudin, KN.; Burant, JC.; Millam, JM.; Iyengar, SS.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, GA.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, JE.; Hratchian, HP.; Cross, JB.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, RE.; Yazyev, O.; Austin, AJ.; Cammi, R.; Pomelli, C.; Ochterski, JW.; Ayala, PY.; Morokuma, K.; Voth, GA.; Salvador, P.; Dannenberg, JJ.; Zakrzewski, VG.; Dapprich, S.; Daniels, AD.; Strain, MC.; Farkas, O.; Malick, DK.; Rabuck, AD.; Raghavachari, K.; Foresman, JB.; Ortiz, JV.; Cui, Q.; Baboul, AG.; Clifford, S.; Cioslowski, J.; Stefanov, BB.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, RL.; Fox, DJ.; Keith, T.; Al-Laham, MA.; Peng, CY.; Nanayakkara, A.; Challacombe, M.; Gill, PMW.; Johnson, B.; Chen, W.; Wong, MW.; Gonzalez, C.; Pople, JA. Gaussian 03, revision c.02. Gaussian, Inc; Wallingford CT: 2004.
48. Aqvist J. Ion water interaction potentials derived from free-energy perturbation simulations. *J Phys Chem.* 1990; 94:8021–8024.
49. Jorgensen WL, Chandrasekhar J, Madura JD, Impey RW, Klein ML. Comparison of simple potential functions for simulating liquid water. *J Chem Phys.* 1983; 79:926–935.
50. Darden T, York D, Pedersen L. Particle mesh ewald - an n.Log(n) method for ewald sums in large systems. *J Chem Phys.* 1993; 98:10089–10092.
51. Allen, MP.; Tildesley, DJ. Computer simulation of liquids. Clarendon Press; Oxford, U.K: 1987.

52. Torrie GM, Valleau JP. Non-physical sampling distributions in monte-carlo free-energy estimation - umbrella sampling. *J Comput Phys.* 1977; 23:187–199.
53. Grossfield, A. Wham: The weighted histogram analysis method. 1.6d.

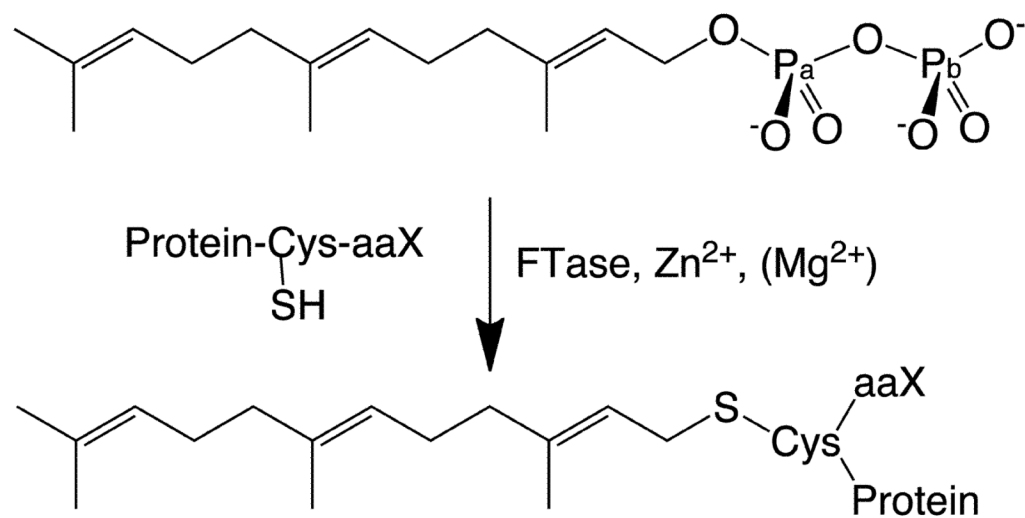


Figure 1.
The FTase catalyzed farnesylation reaction.

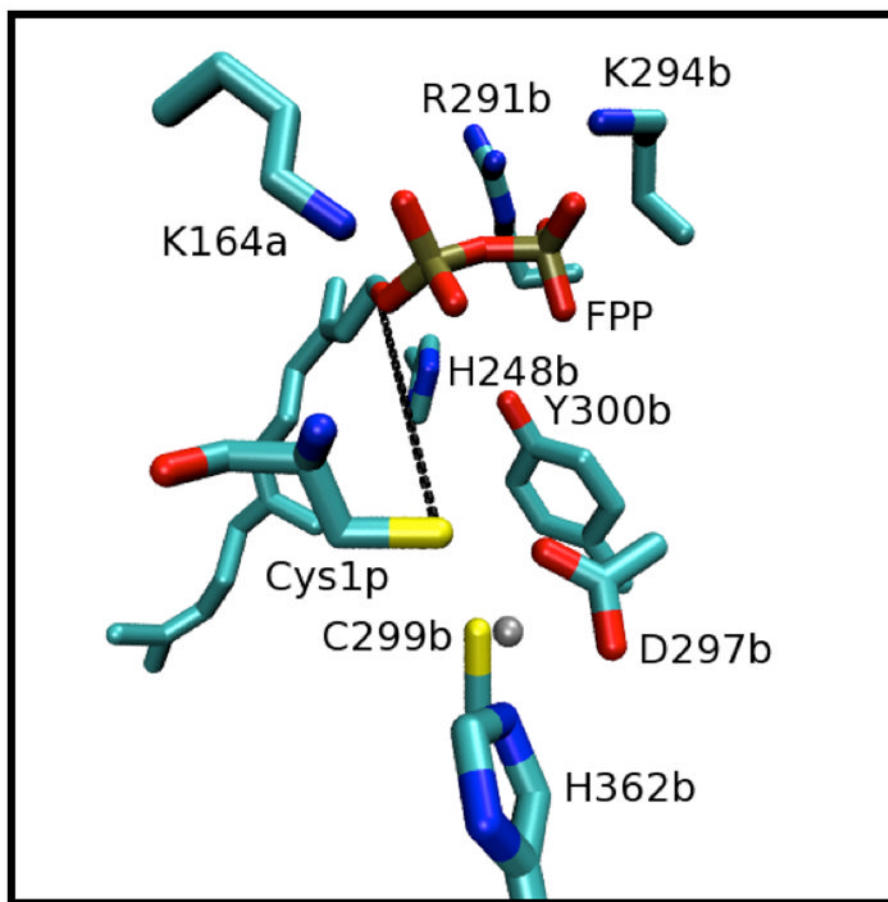


Figure 2. Snapshot of the FTase active site from the initial structure. Zinc is represented as a grey sphere. The black dashed line connects the two reactive atoms, C₁ of FPP and S_γ of the cysteine peptide (Cys1p) and is greater than 7Å in length. Important side chain amino acids are also labeled. Residues in the α and β subunit are labeled with a and b, *e.g.*, K164_α and K294_β are labeled as K164_a and K294_b, separately.

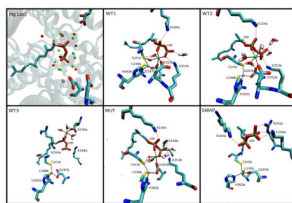


Figure 3.

Snapshots of the FTase active site and important hydrogen bond networks taken from different models. Mg^{2+} (in green) and Zn^{2+} (in grey) ligand environments are shown as black dashed lines while hydrogen bonds are labeled as red dashed lines. Top left: the 10 initial Mg^{2+} placements; top middle: WT1; top right: WT2; bottom left: WT3; bottom middle: MUT; bottom right: SWAP.

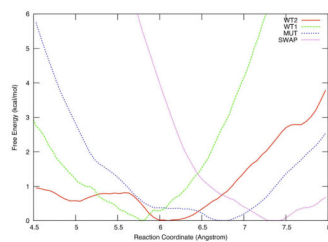


Figure 4. Calculated free energy profiles for the WT1, WT2, MUT and SWAP systems.