An interior water is essential for maintaining the structure of FKBP12

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Introduction

Water is involved in multiple aspects of protein stability and structure, functioning both as a solvent and as a small, strongly-interacting molecule. Desolvation of hydrophobic surface is a driving force in protein folding, leading to the hydrophobic interiors and hydrophilic exteriors observed among globular proteins [1]. Folding to compact structures, however, necessitates the burial of polar main chain nitrogens and oxygens, which as a result often participate in intramolecular hydrogen bonding present in α-helices and β-sheets. The hydrogen bonding needs of main chain groups not involved in these secondary structures, as well as those of buried polar side chains, are often satisfied by buried water molecules [2-6], which may be structurally well-resolved [5, 7]. Buried water molecules and their interactions with the protein have been examined using database analyses, computer simulations, as well as mutational, biochemical, spectroscopic and structural studies [6, 8-16]. Internal water molecules are important components of protein structure and likely play roles in proteins flexibility, folding, and stability of a protein [14, 17-19].

Internal cavities, sometimes called “packing defects”, are common in proteins and can destabilize the structure by an amount that increases with the cavity size [20]. Some of these cavities are occupied by water molecules, which can then stabilize structure by making favorable interactions with the protein, e.g. hydrogen bonds and van der Waals contacts [21]. Experimental studies using cavity inducing mutations have shown that a bound water molecules contribute approximately -2 kcal/mol toward thermal stability [14]. However, there are also entropic costs associated with trapping water molecules, with a penalty of approximately 2 kcal/mol to the free energy [22]. Thus, on average internal waters may be considered energy-neutral. Although thermodynamic studies are highly informative and crucial to ultimately understand the energetics of protein-water interaction, they usually do not provide direct

information on the structural roles of buried waters. For one, the thermostability of a protein alone is not an indicator of whether the protein structure will change in any significant way when mutations are introduced around buried water molecules [23, 24]. Also, some studies have suggested that the binding of interior water molecules may be accompanied by an increase in protein flexibility [25, 26], while others have found that binding a water makes the protein more rigid [27]. These examples highlight the variable nature of protein-water interactions and the need to perform independent, complementary studies to fully characterize the structural roles of buried waters.

We have previously reported the results of molecular dynamics studies of FKBP12 (Fig. 1a). The protein is the target of the immunosuppressant FK506 and belongs to a major class of peptidyl prolyl isomerases that catalyze the cis-trans isomeration of proline [6]. FKBP12 contains a buried solvent molecule Wat3 (which was referred to as Wat137 in Ref. [6]) that makes three main chain hydrogen bonds to the 50s loop and one side chain hydrogen bond to E60 in the helix. Wat3 is well-resolved in FKBP12 and is conserved in other FKBP12-related proteins including FKBP25 and FKBP52 [28, 29]. The molecular dynamics simulations have suggested that Wat3 plays an essential structural role in modulating the shape of the ligand-binding pocket despite its peripheral location. In particular, the simulation studies have predicted that mutating E60 to an amino acid that does not form a strong hydrogen bond with Wat3 will lead to structural rearrangements in neighboring residues as well as those in the ligand-binding pocket (Fig. 9 in Ref. [6]). Herein we present the structures of wild type FKBP12 and two single point mutants, E60A and E60Q. A comparison of the wild type and mutant structures elucidates the mechanism by which a water hydrogen bond to E60 modulates the structure of the ligand binding pocket. The structural changes observed in the mutants provide an important example that buried water can play a key role in maintaining the structural integrity of a protein apart from its contribution to thermal stability. We also discuss the implications of the structural changes seen in the mutants in the context of perturbed hydrogen bonds to the water molecule.

Discussion

The local protein-water interactions of buried solvent have been examined in many biochemical and structural studies [18, 20, 23, 30, 31]. From these studies, we know that buried waters in the protein core can stabilize structures in an aqueous environment by filling “packing defects” within the protein core. Ordered water molecules may function as structural glue in many ligand-receptor or protein-protein complexes by forming intermolecular hydrogen bonds [32-37]; interfacial waters can increase the complementarity of the contacting surfaces. In addition, some water molecules serve highly specialized functional roles. For example, in some enzymes buried waters can help define substrate specificity and facilitate catalysis [24, 38-42]. Statistical analyses of protein crystal structures have shown that internal water molecules are not evenly distributed but rather clustered around regions without secondary structure in order to satisfy the hydrogen bonding needs of buried polar atoms [5, 6, 43]. The study presented here provides evidence that interior water molecules also play a role in maintaining the structural integrity of a protein, in part by forming strong directional interactions via hydrogen bonds.

Wat3 in FKBP12 is well removed from the binding pocket and is not directly involved in ligand binding. Nevertheless, the water appears to be important for the structural integrity of FKBP12 by ensuring that W59 adopts the "correct" conformation consistent with ligand binding. The simulation studies and the crystallographic structures together suggest a plausible mechanism by which Wat3 imposes this structural imprint on the ligand binding pocket (Fig. 4b): The loss of a hydrogen bond between Wat3 and E60 results in a shift of the helix by 1.1 – 1.8 Å perpendicular to its helical axis, pushing W59 on the interior face of the helix against the buried residue F99 on the opposite wall of the ligand binding pocket. The resultant steric clash is resolved through a rotation of the W59 side chain and other structural adjustments in the neighboring side chains. A model of FK506 superimposed on the mutant structures shows the W59 side chain overlapping with the bound ligand. Thus, Wat3 dictates the conformational distribution of distant side chains, some with striking functional consequences.

The manner by which Wat3 impose conformational constraints on structure is reminiscent of some metal ions, e.g. Ca^{2+} and Zn^{2+}, that are known to play important structural roles through strong, noncovalent, directional interactions with neighboring polar atoms [44, 45]. To examine the importance of directionality in protein-water interaction, we introduced a mutation at position 60 that alters the hydrogen bonding environment about Wat3. E60Q is a conservative mutation sterically. Furthermore, considering that Wat3 can easily rotate about its remaining hydrogen bonds, we did not anticipate the substitution of a carboxyl oxygen with an amide nitrogen in E60Q would result in any significant structural change in the protein. Yet, the structural rearrangement in E60Q within the 50s loop bespeaks a different story, in which the substitution of a single hydrogen bonding atom induces a significant rearrangement in the local structure. To wit, the residues 52 and 53 undergo a conformational transition from a left handed α-helical conformation to an extended conformation (Fig. 2b). The fact that a similar rearrangement is observed in the S50 loop for E60A is likely due to the presence of a second highly coordinated water molecule that acts as a surrogate for one of the terminal oxygens of E60. The incorporation of Wat3 in FKBP12 suggests that a buried water molecule may be used to coordinate side chains and to stabilize a target global configuration. The elucidation of the role of Wat3 in FKBP12 thus provides strong evidence that water molecules can serve to promote the integrity, as well as the stability, of protein structure.

A previous database study has found that interior water molecules are likely to form multiple main-chain hydrogen bonds to residues without secondary structure [6]. Wat3 forms part of a network of interacting residues that include the 50s loop (to which Wat3 is anchored through hydrogen bonds), the residues within the N-terminal half of the helix (e.g. W59 and E60), and V55 and F99, whose conformations change in response to the rotation of the W59 side chain. The high level of coupling between Wat3 and protein is also supported in part by its low B-factor (7.7 $Å^2$ vs. 8.0 $Å^2$ for all protein atoms and 19.3 $Å^2$ for all water molecules). The "depth" of the network, spanning several "layers" of residues around the water suggests that the relationship of this water to the protein is qualitatively different from that of water molecules whose principal contribution is thermostabilization. While a strategically positioned internal solvent molecule can qualitatively change the structural and functional properties of the protein, currently we are unable to manipulate buried solvent molecules at will. This has led to a situation where we are unable to generate a large number of systematically varied mutants needed to deduce the underlying physical relationships between protein and structural water molecules.

Notwithstanding, our findings suggest that molecular simulation may be used prior to, as well as in conjunction with, conducting an experimental test to provide important insight into the structural roles of interior water molecules. We expect a combined approach such as presented here to be particularly relevant to address questions that are difficult to observe experimentally, e.g. tracking the movement of single water molecules with atomic precision.

The simulation and structural studies of FKBP12 also help evaluate the current status of computer modeling of bound waters in proteins. Time-resolved spectroscopic studies have confirmed that even water molecules buried deep in the core can exchange with the bulk solvent within nanoseconds to microseconds [46]. We similarly observe Wat3 escaping into the solvent within the 12 nsec simulations, in agreement with the estimated values of residence time for highly dynamic water molecules. The crystallographic structures of the FKBP12 mutants also confirm the predicted conformational role of Wat3, including the side chain dihedral angle changes in W59. However, there are also discrepancies between prediction and observation. Notably, long-range hydrogen bond between G58 NH and Y80 CO was observed to be lost intermittently during the simulation of E60A but was however intact in the experimental structure. Similarly, the main chain conformational change in K52 and Q53 was seen during one of two independent simulations of E60A, but is not seen in the final crystal structure. These latter discrepancies, however, appear to be issues of total computational time rather than methodology [47], and suggest that longer and multiple independent simulations may be necessary to obtain an accurate picture of the impact of structural water. The close agreement between simulation and structure, when it exists, hints that explicit polarization effects need not be modeled in order to obtain quantitatively accurate results—at least in our limited application. They may yet play more prominent roles when computing the free energies of coordinating buried water molecules [26].

A predictive understanding water-mediated long-range interaction is particularly important for efforts in protein folding and design. A modified Hamiltonian with an explicit term to account for protein-water interaction has been shown to improve the accuracy of structure prediction [48]. Structural waters have been modeled using solvated rotamers with some success [49]. While the agreement between simulation and experiment reported herein is encouraging, this success also does not yet tell us how to incorporate water molecules systematically during protein design. For example, two proteins related to FKBP12 (PDB codes 1FD9 and 1PBK) do not contain a buried solvent molecule, but the ligand binding pocket in each still resembles that of FKBP12 [28, 50]. In each structure, however, the identity and arrangement of the residues in the 50s loop and the helix have been altered to produce a helix with a missing main chain hydrogen bond between residues equivalent to E60 and A64. Thus, nature has found two independent solutions to achieve the same structural goals, one that involves a structural water and the other that does not. Once we understand the protein-water interaction better, we may hope to replicate some of these success stories in the laboratory. The complementary use of molecular dynamics simulations and crystallographic studies discussed in this paper presents a small step towards achieving that goal.

The role of water in protein folding and stability continues to be a subject of great interest. We investigated the specificity of structural coupling between buried water and protein in order to test how accurately bound water reproduce structural properties of protein atoms. This is apart

from the contribution of buried water molecules to thermal stability, which may involve both enthalpic and entropic components, or their involvement in catalysis, in which bound water molecules sometimes function as pseudo-co-factors. In order to be considered an extension of protein structure, water molecules must participate in interactions that are both specific and unique, as these are characteristics one would look for in protein side chain atoms involved in intramolecular hydrogen bonding interactions. We established that Wat3 qualifies as an integral part of the FKBP12 structure by introducing a highly focused perturbation to the network of interaction around it and demonstrating that this results in a remodeling of the distant ligand binding pocket. In the process, we have also examined the accuracy of the predictions based on molecular dynamics simulations. Comparing the simulation results with crystal structures shows that simulations can predict some events with atomic resolution while grossly missing other properties, although the latter are likely due to limited sampling rather than fundamental flaws in the calculations.

Figure and Table Captions

Figure 1

a. FKBP12 is comprised of a five-stranded anti-parallel β sheet wrapped around a short α helix. The 50s loop hydrogen bonded to the conserved structural water Wat3 is highlighted.
b. The side chains are superimposed on the electron density at 2.5σ.

Figure 2

a. The hydrogen bonding network around Wat3 in i) wild type, ii) E60Q, iii) E60A, and iv) simulated structure of E60Q. E60 adopts two alternate conformations in wild type, which differ from each other by a rotation about one of the terminal oxygens. The coordinates of Oε1 are approximately the same in both conformations, thus permitting it to form a stable hydrogen bond with Wat3. The second "B" conformation is shown in yellow.
b. Ramachandran plots for K52 and Q53 from simulations of wild type (left) and E60Q (right) structures. The yellow and red dots indicated by arrows correspond to the dihedral angles observed in the final crystal structures.

Figure 3

a. In the mutants, the N-terminus of the helix moves toward the center of the protein (inset), with the main chain atoms from W59 and A60 moving by 1.28 – 1.74 Å (dotted lines).
b. The ligand binding pocket of the E60Q mutant (outlined as oval). The side chain of W59 rotates to a new conformation to avoid steric clash with F99. The conformation of V55 and F99 changes to optimize van der Waals contacts with W59 in the new conformation.

Figure 4

a. FK506 (from PDB 1FKF) modeled in the ligand binding pocket of wild type (violet) and E60Q (cyan) FKBP12. The rotation of W59 leads to a steric clash between its side chain and the docked FK506.
b. The network of interacting amino acids and Wat3 in FKBP12.

Table 1

A summary of data collection and final statistics

Figures

Figure 1

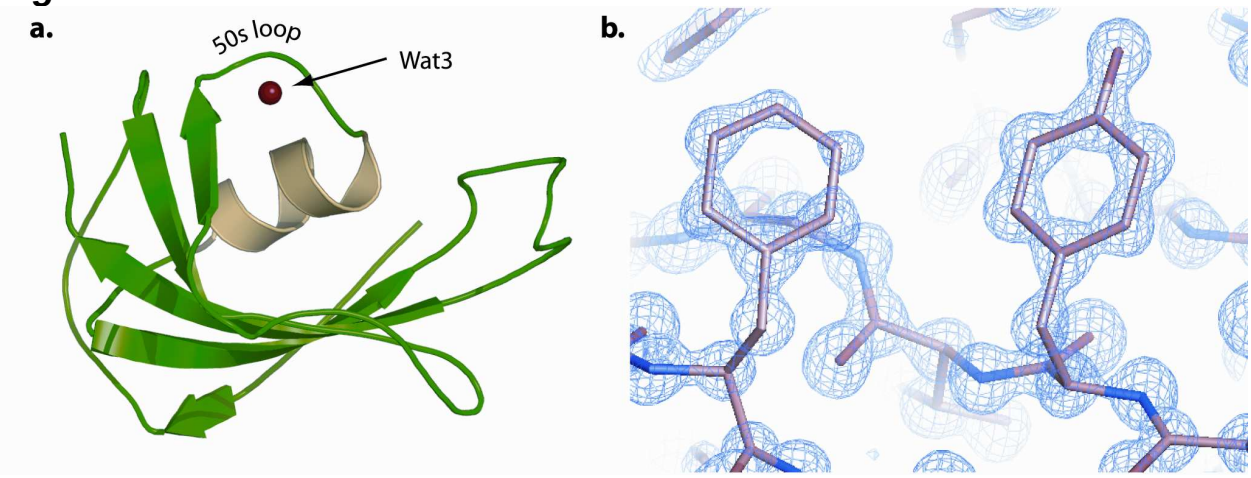


Figure 2

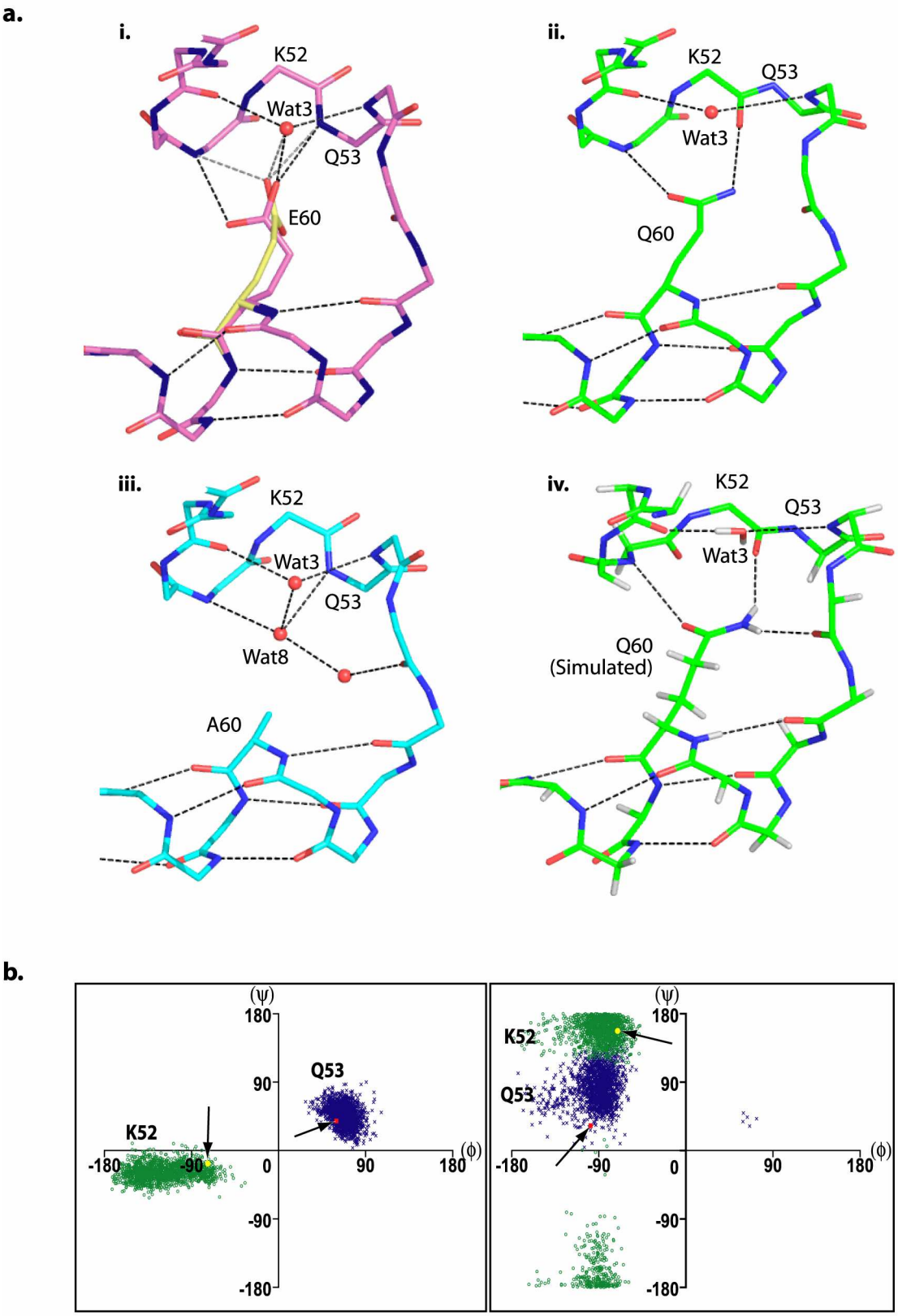

a.
i.
K52
Wat3
Q53
E60
ii.
K52
Q53
Wat3
Q60
iii.
K52
Wat3
Q53
Wat8
A60
iv.
K52
Q53
Wat3
Q60
(Simulated)
b.
(ψ)
180
90
-90
-180
Q53
K52
-180
-90
0
90
180
(ϕ)
(ψ)
180
90
-90
-180
K52
Q53
-180
-90
0
90
180
(ϕ)

Figure 3

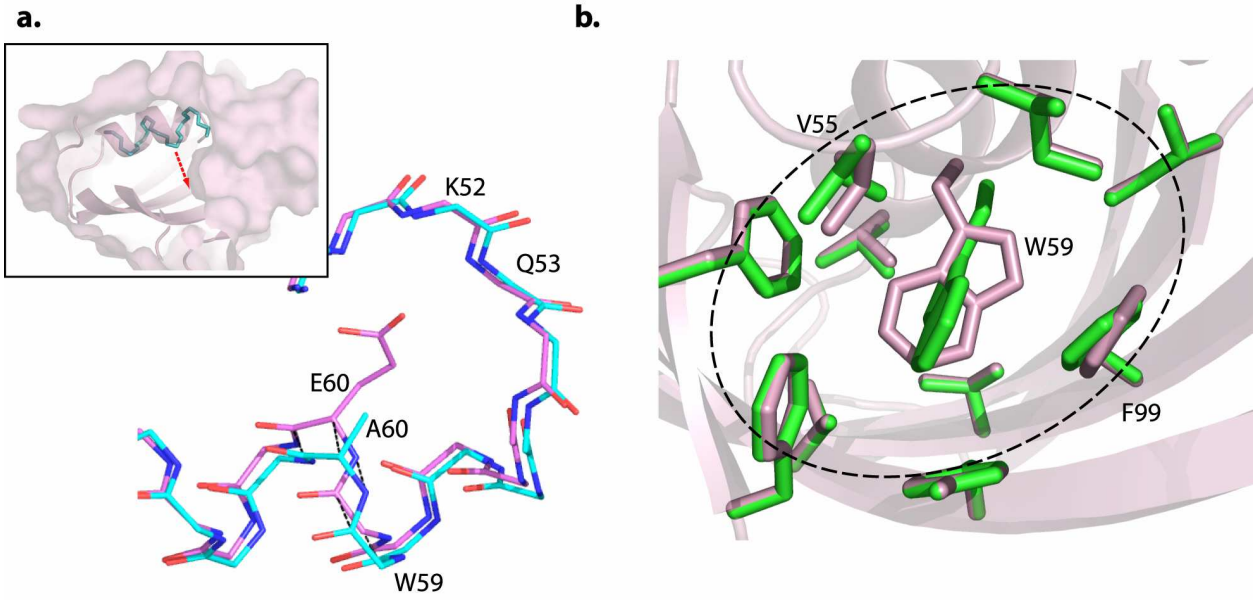
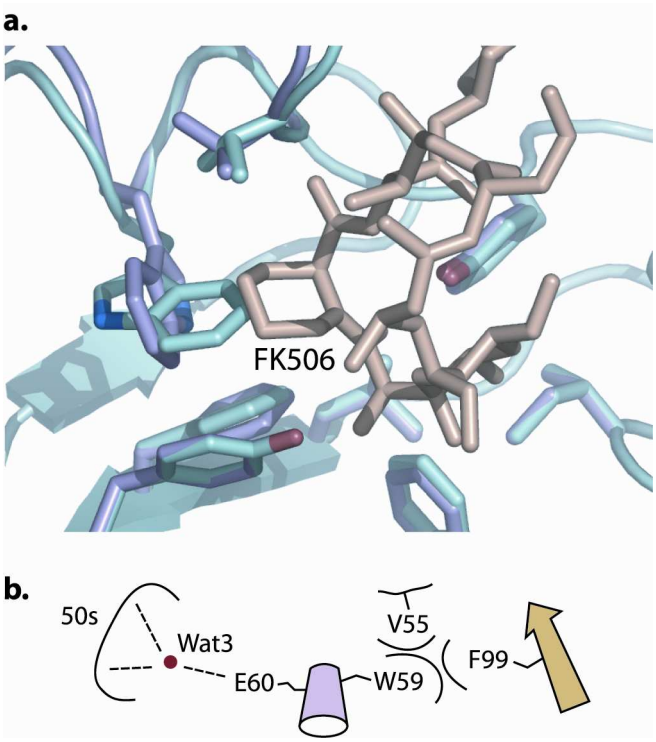


Figure 4



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