Supplemental Information

Visualizing the Tunnel in Tryptophan Synthase with Crystallography: Insights into a Selective Filter for Accommodating Indole and Rejecting Water

Eduardo Hilario[a], Bethany G. Caulkins[b], Yu-Ming M. Huang[b,1], Wanli You[b], Chia-En A. Chang[b], Leonard J. Mueller[b,*], Michael F. Dunn[a,*], Li Fan[a,*]

[1]Department of Biochemistry, University of California at Riverside, Riverside CA 92521, USA

[2]Department of Chemistry, University of California at Riverside, Riverside CA 92521, USA

[*]Corresponding author

The Supplemental Information provides more technically detailed descriptions of the crystallographic work regarding the following: (1) refinement of electron density for the molecule bound to site F6-1 in the β-subunit of TS for the model, PDB ID: 2CLF; (2) comparison of the conformations of TS containing one, two, or three molecules of F6 bound to the αβ-dimeric units of models, PDB ID: 5BW6, 4Y6G, 4WX2, and 4ZQC; and (3) protein-water interactions in the tunnel.

The orientation and position of the F6-2 ligand in PDB ID models 4WX2, 4ZQC, 4Y6G, and 5BW6 matches well with the same molecule in PDB ID models 2CLE and 2CLF (Figure 1d). The F6-3 ligand was not observed in PDB ID models 2CLE, 2CLF[1] or 5BW6. Under soaking conditions with lower F6 concentrations (1 mM) we also obtained a crystal structure (PDB ID: 4Y6G) with F6 bound to two loci, corresponding to the locations and orientations of F6-1 and F6-2. However, lowering the soaking time at the same concentration we obtained a crystal

structure (PDB ID: 5BW6) with F6 molecule (F6-2) bound exclusively in the α-site.

The flipped F6-1 ligand in model PDB ID: 2CLF. The PDB ID: 4WX2 crystal structure was solved by molecular replacement (MR) using the search model PDB ID: 4KKX[2]. Prior to the MR step, the coordinate file of PDB ID: 4KKX was edited and the solvent molecules and ligands were removed except for the F6 molecule located in the α-site. The MR solution was briefly restrained, refined, and two positive and well-defined electron densities were identified in the 2Fo-Fc map. Based on their sizes and shapes, another two F6 molecules were added to the crystal structure: the first close to PLP in the β-site, and the second close to the F6 ligand in the α-site. Surprisingly, in model PDB ID: 4WX2, we noticed the F6 molecule in the β-site (F6-1) was flipped by approximately 180° when compared with the corresponding molecule in model PDB ID 2CLF. Later, the crystal structures PDB IDs: 5BW6, 4Y6G, and 4ZQC were solved by MR using as the search model, PDB ID: 4WX2, without the solvent and F6 molecules. The MR solution was extensively refined, and the F6-1 molecule in PDB ID: 4Y6G and 4ZQC was found to have the same orientation as in model PDB ID: 4WX2. Based on this finding, we decided to investigate the orientation of the corresponding molecule in PDB ID: 2CLF[3], since we were otherwise confronted with the possibility of a dual orientation of the F6 ligand at the β-site. Model PDB ID: 2CLF[3] was refined at 1.70 Å resolution using the software CNS 1.1.[3] The R and R_{free} values are 17.6 and 19.6%, respectively, using a randomly selected 5.0% of total reflections. We were interested in re-refining this model, but with rotation of the F6-1 molecule to the orientation we observed in our models PDB IDs: 4WX2, 4ZQC, and 4Y6G. In order to re-refine model PDB ID: 2CLF (rr2CLF), the coordinate file and the structure factor (SF) file of model PDB ID: 2CLF were downloaded from the PDB web-site and then uploaded in the Structure Factor Conversion and Validation (sf-valid) version 2.03 (http://sf-tool.wwpdb.org/) to

convert the SF file from the mmCIF format to MTZ format, using 5.0% of the reflection data (free R) for cross-validation. Then, the F6-1 ligand (molecule B1396F6F) in the β-site of model PDB ID: 2CLF was rotated about 180° from the original orientation. After several rounds of TLS with restrained refinement cycles in Refmac5[4] and minimal structure adjustments in Coot[5], both R (14.4%) and R_{free} (16.4%) values decreased significantly. For additional F6 ligand comparisons, we listed the B-factors for all F6 atoms, except hydrogens, in model rr2CLF and PDB ID models 2CLF, 5BW6, 4Y6G, 4WX2, and 4ZQC (Table S2). For the F6-1 molecule in the β-site, the B-factor mean value in model PDB ID: 2CLF is slightly higher (33.56 $Å^2$) when compared with the same molecule in models PDB IDs: 4WX2 (26.79 $Å^2$), 4ZQC (18.79 $Å^2$), and 4Y6G (30.5 $Å^2$). After the re-refinement of the crystal structure PDB ID: 2CLF, the B-factor mean value of the F6-1 molecule decreased 17.8% (to 27.59 $Å^2$) when compared with the same molecule in model PDB ID: 2CLF (33.56 $Å^2$). For the ligand F6-2 in the α-site, model PDB ID: 4Y6G has a slightly higher B-factor mean value (34.74 $Å^2$) when compared with models PDB ID: 2CLF (32.51 $Å^2$) and PDB ID: 4WX2 (30.31). In model rr2CLF, the B-factor mean value (29.66 $Å^2$) decreased 8.8% when compared with same ligand in model PDB ID: 2CLF. The B-factor mean values of F6-3 molecule in PDB ID: 4WX2 (45.94 $Å^2$) and 4ZQC (27.60 $Å^2$) are high mostly due to their higher mobility in the αβ-subunit interface region. Fig. 3a-c shows sections of the electron density maps in the β-site contoured at 1.20 RMS from a 2Fo-Fc map of models PDB ID: 4WX2 (molecule βF6F404), 4ZQC (molecule βF6F402), and 4Y6G (molecule βF6F402), respectively, at 1.75, 1.54, and 1.65 Å resolution. The electron density of the F6-1 molecule is strong, well defined, and covers the entire extension of the F6 ligand; strongly suggesting the ligand is properly oriented in the β-site. Model PDB ID: 2CLF displays a weak and disconnected electron density for the corresponding molecule (molecule βF6F1396) in the

2Fo-Fc electron density contoured at 1.2 rms (Fig. 3d). In the PDB ID: 2CLF, a connected electron density that covers the entire F6-1 molecule is observed when the 2Fo-Fc electron density map was contoured at 0.5 rms. At this contoured map level, it is possible to detect a suggestive and circular shaped electron density (red hexagon as shown in Fig. 3e) belonging to the phenyl group of the F6 ligand and an additional electron density belonging to the O14 in the same ligand (=O in Fig. 3e). After the re-refinement of the PDB ID: 2CLF model, a better electron density that covers the entire ligand was observed for the 2Fo-Fc electron density map contoured at 0.7 rms (Fig. 3f); the rms error in the density was minimized, suggesting the F6-1 molecule was properly fitted and refined into the 2Fo-Fc electron density.

Conformational Differences at the αβ Subunit Interface. Noticeably, on one side of the tunnel near the αβ-interface, the C^{α} atoms of β-site residues Ile157, His160, Gly162, Ser163, Leu166, Lys167, Ala169, and Cys170 at the loop of the COMM domain clamp down about 0.7, 0.9, 1.5, 1.2, 1.5, 0.8 Å, respectively, giving significantly larger shifts than the RMSD of 0.338 Å. On the other side of the tunnel, the C^{α} atoms of β-site residues Gln293, Ile294, Glu295, Glu296, Ser297 on the β-hairpin (residues β288-β300) also shift toward the tunnel by 0.6, 0.7, 1.6, and 1.1 Å, respectively. Interestingly, loop αL2 is lifted upward to the α-active site in the PDB ID: 4WX2 structure with the C^{α} atoms of α-site residues Ser55, Asp56, Pro57, Leu58, Ala59, and Gly61 shifted 0.6, 1.6, 1.8, 1.3, 0.9, and 0.7 Å, respectively. This lifting on loop αL2 is not caused by the binding of F6-2 at the αβ-interface since the PDB ID: 4Y6G structure without this additional F6 molecule has the same lifting of loop αL2. However, the binding of F6-2 at the αβ-interface impacts the tunnel by reducing the clamping down of the loop in the COMM domain. Without this molecule in the PDB ID: 4Y6G structure, the C^{α} atoms of β-site residues His160, Gly162, Ser163, Lys167, and Asn171 clamp down further, 0.8, 0.7, 0.8, 0.6, 0.5

Å, respectively, when compared with the same C^{α} atoms in the PDB ID: 4WX2 structure. In addition, β-site residues Glu109, Leu166, Cys170, Asn171, Glu296, Ser297, Asp305, and Phe306 of PDB ID: 2CLE have different rotameric conformations than those observed in PDB IDs: 4Y6G, 4ZQC, and 4WX2 containing the bound F6 ligand in the β-site. The most remarkable side-chain change is at residue βPhe280, whose aromatic side chain protrudes into the tunnel in PDB IDs: 2CLE and 5BW6, but turns away in PDB IDs: 4Y6G, 4ZQC, and 4WX2 and is stacked between residues βTyr279 and βPhe306. Residue βPhe280 in PDB ID: 5BW6 has an alternative rotameric conformation (10% of residue occupancy) due to a strong positive electron density in the Fo-Fc map when this alternative conformation is absent from PDB ID 5BW6 model. However, the alternative conformation is similar to that observed in the PDB IDs: 4Y6G, 4ZQC, 4WX2, and 2CLF crystal structures.

The Binding of F6 to Tryptophan Synthase. The F6 ligand is an amphipathic compound composed of 21 heavy atoms ($C_{10}H_{11}F_3NO_6P$). The hydrophobic head located at one end of the molecule consists of the trifluoromethoxy group (F-9-11, C-8, O-7) and phenyl atoms (C-1-6, H-2-3, and H-5-6) followed by the polar tail composed of the carboxamido group (C-12, O-14 and N-13) and the ethyl (C-15-16) phosphoryl group (O-17, P-18, O-19, H-19, O-20-21, and H-12). The contact distances and the atomic interactions between F6 ligand, solvent molecules, and protein residues are listed in Table S1A-C.

F6-Protein and Water Interactions. In models PDB ID: 4WX2 (molecule βF6F404), PDB ID 4ZQC (molecule βF6F402), and PDB ID: 4Y6G (molecule βF6F402), the binding site for F6-1 resides almost completely within the tunnel connecting the β-site with the α-site. In both models, the hydrophobic head group makes weak contacts within the β-subunit with Glu109, Cys170, Leu174, Tyr186, Leu188, Pro194, Phe280, Phe306, and with a single water molecule; molecule

β894 in PDB ID: 4WX2 and molecule β960 in PDB ID: 4Y6G. In all three models, the polar tail makes extensive hydrogen bonding contacts with the amino acid residues βLys87, βGlu109, βThr110, βGly111, βAla112, βGln114, βHis115, βThr190, the PLP C-4' carbon, and neighboring water molecules β641, β667, and β874 (in PDB ID: 4WX2); water molecules β515, β521, and β571 (in PDB ID: 4ZQC); and water molecules β717, β892, β965, and β989 (in PDB ID: 4Y6G) (see Table S1A for additional bond length details). Amino acid residues form part of the tunnel wall in the β-subunit extending from βLys87 and βGln114 in the β-catalytic site to the side chains of βLeu174 and βPhe280 approximately 18 Å away. Two of the waters are hydrogen bonded to the F6 phosphoryl group; water molecules β667 and β874 in PDB ID: 4WX2, molecules β515, β521; water molecule β571 in PDB ID: 4ZQC; and water molecules β892 and β965 in PDB ID: 4Y6G. Respectively in PDB ID models 4WX2, 4ZQC, and 4Y6G, one (β641), one (β709), or two (β717 and β989) water molecule(s) are hydrogen bonded to the oxygen of the F6 carboxamide.

The F6-2 ligand (molecule αF6F301) in PDB IDs: 4WX2, 4ZQC, 4Y6G, and 5BW6 and the F6-3 ligand (molecule αF6F302) in PDB IDs: 4WX2, 4ZQC, and 4Y6G are bound to sites within the α-subunit near the αβ-subunit interface and hydrogen-bonded to each other (see below). In both models, weak contacts between protein residues and the hydrophobic head of F6-2 involve α-subunit residues Ala59, Asp60, Leu100, Leu127, Ala129, Ile153, Tyr175, Phe212, and the β-subunit residue Pro18. Three highly conserved water molecules make weak contacts with the hydrophobic trifluoromethoxy group of the F6-2 molecule; waters α451, α596, and β647 in PDB ID: 4Y6G; water α437 and the split water β626A/B in PDB ID: 4WX2; waters α541, α675, and β801 in PDB ID: 4ZQC; waters α598, α635, and α728 in PDB ID: 5BW6 (see Table S1B for details). The α-subunit residues Glu49, Tyr175, Gly213, Gly234, and Ser235 form hydrogen-

bonds to polar atoms on F6-2. Two highly conserved water molecules form hydrogen-bonds with the phosphoryl group on the F6-2 ligand; waters α458 and α523 in PDB ID: 4WX2; waters α415 and α418 in PDB ID: 4ZQC, and waters α469 and α501 in PDB ID: 4Y6G; and waters α404 and α413 in PDB ID: 5BW6 (see Table S1B for details).

Molecule F6-3 (αF6F302) in PDB ID: 4WX2 and 4ZQC binds within the α-catalytic site at several loci in common with the substrate IGP binding site. Weak contacts are made between the hydrophobic head of F6-3 and α-subunit resides Leu58 and Ala59 and β-subunit residues Pro18, Ile20, Leu21, Arg175, and Ser178. The α-subunit residues Ala59, Asp60, Gly61, Ile64, Ser235, and waters α526, α601-603 in PDB ID: 4WX2 or waters α433 and α464 in PDB ID: 4ZQC all make hydrogen bonds with F6-3, and, as mentioned above, F6-2 and F6-3 are hydrogen-bonded to each other through their phosphoryl group oxygens (O-19 of F6-3 to O-20 of F6-2 in PDB ID: 4WX2 and O-19 of F6-3 to O-21 of F6-2 in PDB ID: 4ZQC) and between the phosphoryl O-19 of F6-3 and the carboxamide nitrogen of F6-2 (N-13); these interactions require that one of these phosphoryl oxygens is protonated (see Table S1C for additional details). The position of F6-1 in the tunnel of the PDB IDs: 4WX2, 4ZQC, and 4Y6G overlaps with the position assigned to the F6 molecule (β1396) in PDB ID: 2CLF (Fig. 2 and Fig. 3). However, the orientation of the F6-1 molecule is flipped by approximately 180° in PDB IDs: 4WX2, 4ZQC, and 4Y6G in comparison to that assigned to F6 molecule in the PDB ID: 2CLF. We chose the flipped orientation for two reasons: (1) this orientation gives a significantly better fit to the electron density map; (2) the flipped orientation places the hydrophobic head of F6-1 into a highly nonpolar region of the tunnel and the carboxamide N into hydrogen-bonding distance of βGlu109, while the polar phosphoryl group occupies a polar subsite of the β-catalytic site designed for the binding of the carboxylate moieties of substrates L-Ser and L-Trp. The orientation and position of the F6-2

ligand in PDB IDs: 4WX2, 4ZQC, 4Y6G, and 5BW6 matches well in comparison to that assigned to the F6 molecules in PDB IDs: 2CLE and 2CLF. Molecule F6-3 was not observed in PDB IDs: 4Y6G, 5BW6, 2CLE, and 2CLF. Under soaking conditions with lower F6 concentrations we also obtained crystals (PDB ID: 4Y6G) with F6 bound to two loci, corresponding to the location and orientation of F6-1 and F6-2 in our TS crystal structures. However, lowering the soaking time in buffer containing 1mM F6 ligand we obtained a crystal structure (PDB ID: 5BW6) with F6 bound only to the alpha-site.

Table S1A. Interactions of F6-1 ligand in the β-site with protein atoms and solvent molecules. The structures PDB IDs: 4WX2, 4ZQC, and 4Y6G are compared and distances for the same atom pairs are listed in the same row. Bond lengths are given in Angstroms (Å).

	4WX2			**4ZQC**			**4Y6G**	
Ligand atom	**Atom/ Residue**	**Bond length**	**Ligand atom**	**Atom/ Residue**	**Bond length**	**Ligand atom**	**Atom/ Residue**	**Bond length**
C2	OH/βTyr186	3.43	C2	OH/βTyr186	3.38	C2	OH/βTyr186	3.49
C2	SG/βCys170	3.45	C2	SG/βCys170	3.46	C2	SG/βCys170	3.44
C2	CD2/βLeu188	3.49	C2	CD2/βLeu188	3.59	C2	CD2/βLeu188	3.47
C3	OE2/βGlu109	3.05	C3	OE2/βGlu109	3.26	C3	OE2/βGlu109	3.13
C3	SG/βCys170	3.40	C3	SG/βCys170	3.32	C3	SG/βCys170	3.36
C5	CE1/βPhe306	3.20	C5	CE1/βPhe306	3.18	C5	CE1/βPhe306	3.33
O7	CD1/βLeu188	3.80	O7	CD1/βLeu188	3.66	O7	CD1/βLeu188	3.74
F9	CD1/βLeu174	3.73	F10	CD1/βLeu174	3.58	F10	CD1/βLeu174	3.66
F9	O/βPhe280	2.88	F10	O/βPhe280	2.96	F10	O/βPhe280	2.92
F9	CD/βPro194	3.64	F10	CD/βPro194	3.65	F10	CD/βPro194	3.88
F10	β894	3.26				F11	β960	3.34
F10	CB/βCys170	3.47	F11	CB/βCys170	3.52	F11	CB/βCys170	3.46
F10	CD1/βPhe280	3.63	F11	CD1/βPhe280	3.76	F11	CD1/βPhe280	3.68
F11	CD1/βLeu174	3.76	F9	CD1/βLeu174	3.71	F9	CD1/βLeu174	3.73
F11	OH/βTyr186	3.31	F9	OH/βTyr186	3.35	F9	OH/βTyr186	3.30
F11	CD1/βLeu188	3.66	F9	CD1/βLeu188	3.46	F9	CD1/βLeu188	3.73
N13	OE2/βGlu109	2.62	N13	OE2/βGlu109	2.82	N13	OE2/βGlu109	2.72
O14	CG2/βThr190	3.23	O14	CG2/βThr190	3.18	O14	CG2/βThr190	3.48
O14	β641	2.84	O14	β709	2.87	O14	β717	2.87
O17	β667	3.01	O17	β521	3.29	O17	β965	3.10
O19	β667	2.76	O21	β515	2.59			
O19	β874	2.57	O21	β521	2.59	O20	β965	2.84
O19	C4A/βPLP401	3.45	O21	C4A/βPLP401	3.95	O20	C4A/βPLP401	3.56
			O19	β571	2.69	O19	β892(A)	2.56
O20	N/βGly111	2.86	O19	N/βGly111	2.97	O19	N/βGly111	2.80
O20	N/βAla112	3.06	O19	N/βAla112	4.02	O19	N/βAla112	3.08
O21	OG1/βThr110	2.39	O20	OG1/βThr110	2.43	O21	OG1/βThr110	2.24
O21	N/βGly111	3.19	O20	N/βGly111	3.14	O21	N/βGly111	3.28
O21	N/βHis115	3.05	O20	N/βHis115	2.96	O21	N/βHis115	2.97
O21	N/βGln114	3.66	O20	N/βGln114	3.61	O21	N/βGln114	3.46

Table S1B. Interactions of F6-2 ligand in the α-site with protein atoms and solvent molecules. Structures PDB IDs: 4WX2, 4ZQC, 4Y6G, and 5BW6 are compared and distances for the same atom pairs are listed in the same row. Bond lengths are given in Angstroms (Å).

	4WX2			**4ZQC**			**4Y6G**			**5BW6**	
Ligand atom	**Atom/ Residue**	**Bond length**	**Ligand atom**	**Atom/ Residue**	**Bond length**	**Ligand atom**	**Atom/ Residue**	**Bond length**	**Ligand atom**	**Atom/ Residue**	**Bond length**
C3	CE1/αPhe212	4.10	C5	CE1/αPhe212	3.95	C5	CE1/αPhe212	3.77	C5	CE1/αPhe212	3.50
C3	OD1/αAsp60	3.45	C5	OD1/αAsp60	3.69	C5	OD1/αAsp60	3.55	C5	OD1/αAsp60	3.67
C4	CE1/αPhe212	3.84	C4	CE1/αPhe212	3.66	C4	CE1/αPhe212	3.54	C4	CE1/αPhe212	3.47
C5	CE1/αPhe212	3.82	C3	CE1/αPhe212	3.69	C3	CE1/αPhe212	3.60	C3	CE1/αPhe212	3.76
C5	CD2/αLeu100	3.90	C3	C12/αLeu100	4.08	C3	CD1/αLeu100	3.97	C3	CD1/αLeu100	3.84
C2	OD1/αAsp60	3.47	C6	OD1/αAsp60	3.74	C6	OD1/αAsp60	3.78	C6	OD1/αAsp60	3.90
C6	CD2/αLeu100	3.59	C2	CD1/αLeu100	3.71	C2	CD1/αLeu100	3.75	C2	CD1/αLeu100	3.73
O7	CB/αAla59	3.28	O7	CB/αAla59	3.30	O7	CB/αAla59	3.13	O7	CB/αAla59	3.34
F9	CB/αAla59	3.10	F9	CB/αAla59	3.22	F9	CB/αAla59	3.32	F9	CB/αAla59	3.44
F9	O/αAla129	3.20	F9	O/αAla129	3.12	F9	O/αAla129	3.03	F9	O/αAla129	3.02
F9	α437	3.68	F9	α541	3.48	F9	α451	3.34	F9	α635	3.24
F9	CB/βPro18	3.45	F9	CB/βPro18	3.35	F9	CB/βPro18	3.37	F9	CB/βPro18	3.08
F10	α437	3.49	F10	α541	3.29	F10	α451	3.23	F10	α635	2.86
F10	CD2/αLeu127	3.71	F10	CD2/αLeu127	3.49	F10	CD2/αLeu127	3.63	F10	CD2/αLeu127	3.59
F10	CG1/αIle153	3.48	F10	CG1/αIle153	3.35	F10	CG1/αIle153	3.41	F10	CG1/αIle153	3.33
F11	α626B(B)	2.68	F11	α675	2.69	F11	α596	2.74	F11	α598	2.49
F11	α626B(A)	3.70	F11	A801	3.59	F11	β647	3.71	F11	β728	3.35
C12	OH/αTyr175	3.11	C12	OH/αTyr175	3.20	C12	OH/αTyr175	3.10	C12	OH/αTyr175	3.22
N13	O19/αF6-3	2.74	N13	O19/αF6-3	2.76						
O14	OE1/αGlu49	3.35	O14	OE2/αGlu49	3.46	O14	OE2/αGlu49	3.41	O14	OE1/αGlu49	2.72
O14	OE2/αGlu49	2.46	O14	OE1/αGlu49	2.58	O14	OE1/αGlu49	2.57	O14	OE2/αGlu49	3.24
O14	OH/αTyr175	2.58	O14	OH/αTyr175	2.66	O14	OH/αTyr175	2.61	O14	OH/αTyr175	2.57
									O17	α413	3.02
O19	N/αGly213	2.77	O20	N/αGly213	2.76	O21	N/αGly213	2.79	O20	N/αGly213	3.19
									O20	α510	3.21
			O20	α433	2.73						
									O21	α510	2.99
O20	OG/αSer235	2.73	O21	OG/αSer235	2.89	O20	OG/αSer235	2.56	O21	OG/αSer235	2.31
O20	N/αSer235	2.71	O21	N/αSer235	2.74	O20	N/αSer235	2.82	O21	N/αSer235	2.54
O20	O19/αF6-3	2.64	O21	O19/αF6-3	2.47						
O21	N/αGly234	2.89	O21	N/αGly234	2.83	O21	N/αGly234	2.76	O19	N/αGly234	3.41
O21	α523	2.44	O19	α415	2.62	O21	α469	2.62	O19	α404	2.49
O21	α458	2.66	O19	α418	2.73	O21	α501	2.69	O19	α413	2.88

Table S1C. Interactions of F6-3 ligand in the α-site with protein atoms and solvent molecules. The bond length is given in Angstroms (Å). Structures PDB IDs: 4WX2 and 4ZQC are compared and distances for the same atom pairs are listed in the same row. Bond lengths are given in Angstroms (Å).

	4WX2			4ZQC	
Ligand atom	**Atom/ Residue**	**Bond length**	**Ligand atom**	**Atom/ Residue**	**Bond length**
C1	CD1/βIle20	3.55		-	
C2	OG/βSer178	3.71	C6	OG/βSer178	3.51
C3	α602	3.07		-	
C4	O/αAla59	3.12	C4	O/αAla59	3.04
C5	β626	3.17	C3	β675	3.20
C5	O/αAla59	3.17	C3	O/αAla59	3.18
C6	CB/αAla59	3.70	C2	CB/αAla59	3.70
O7	CD1/βIle20	3.42	O7	CG2/βIle20	3.41
O7	OG/βSer178	3.77	O7	OG/βSer178	3.62
F9	CG/αLeu58	2.99	F9	CG/αLeu58	3.38
F9	CD/βPro18	3.52	F9	CD/βPro18	3.48
F10	CD/βPro18	3.77	F10	CD/βPro18	3.55
F10	CD1/βLeu21	2.80	F10	CD1/βLeu21	2.95
F11	CA/βArg175	3.27	F11	CA/βArg175	3.47
F11	OG/βSer178	3.67	F11	OG/βSer178	3.57
C12	O/αAla59	3.05	C12	O/αAla59	2.83
N13	α601	2.97			
O14	O17/αF6-3	2.86	O14	O17/αF6-3	3.05
O14	C2/αF6-2	3.74	O14	C6/αF6-2	3.40
O14	O/αAla59	2.83	O14	O/αAla59	2.91
O14	CE2/αPhe212	2.93	O14	CE2/αPhe212	2.83
O14	CZ/αPhe212	2.80	O14	CZ/αPhe212	2.59
C15	α603	3.14	C15	A433	3.36
O17	C3/αF6-2	3.31	O17	C5/αF6-2	3.18
O19	N13/αF6-2	2.74	O19	N13/αF6-2	2.76
O19	O20/αF6-2	2.64	O19	O21/αF6-2	2.47
O20	OD1/αAsp60	2.61	O21	OD1/αAsp60	2.38
O20	CA/αAsp60	3.16	O21	CA/αAsp60	3.11
O20	N/αGly61	3.12	O21	N/αGly61	3.40
O20	CG1/αIle64	2.98	O21	CG1/αIle64	3.29
O20	CB/αIle64	3.19	O21	CB/αIle64	3.28
O20	α526	3.40	O21	α464	4.11
O21	α526	2.66	O20	α464	2.73
			O20	α433	2.69
O21	N/αGly61	3.57	O20	N/αGly61	3.18
O21	OG/αSer235	3.28	O20	OG/αSer235	3.53

Table S2. Temperature factor (B-factor) comparisons of F6 ligands in TS models PDB IDs: 2CLF, 4Y6G, 4WX2, 4ZQC, and 5BW6. Model rr2CLF is the model re-refined from PDB ID: 2CLF. Crystal structures rr2CLF, and models PDB IDs: 2CLF, 4Y6G, 4WX2, 4ZQC, and 5BW6 were refined, respectively, at 1.70, 1.70, 1.65, 1.75, 1.54, and 1.82 Å resolution.

Model	2CLF	rr2CLF	4Y6G	4WX2	4ZQC	2CLF	rr2CLF	5BW6	4Y6G	4WX2	4ZQC	4WX2	4ZQC
F6 molecule	F6-1	F6-1	F6-1	F6-1	F6-1	F6-2	F6-2	F6-2	F6-2	F6-2	F6-2	F6-3	F6-3
Identity	β1396	β1396	β402	β404	β402	α1269	α1269	α301	α301	α301	α301	α302	α302
Location	β-site	β-site	β-site	β-site	β-site	α-site	α-site	α-site	α-site	α-site	α-site	α-site	α-site
Occupancy (%)	50	50	90	95	80	100	100	70	90	85	100	80	80
R (%)	17.6	14.4	15.8	15.8	13.57	17.6	14.4	16.8	15.8	15.8	13.57	15.8	13.57
R_{free} (%)	19.6	16.4	17.9	18.8	18.66	19.6	16.4	21.4	17.9	18.8	18.66	18.8	18.66
F6 atom						B-factor	(Å^2)						
C1	33.80	26.01	30.19	25.10	17.60	34.23	29.84	37.64	33.82	28.34	17.00	46.63	30.79
C2	33.24	25.73	28.96	23.71	19.76	33.17	27.86	37.39	33.32	26.42	16.77	47.06	27.83
C3	32.52	26.85	28.14	25.70	17.32	32.35	27.67	34.82	30.67	28.14	13.44	51.04	29.99
C4	32.01	25.92	26.91	24.58	17.68	31.53	27.61	35.13	31.93	26.13	13.61	50.79	31.13
C5	33.27	26.03	28.10	26.40	16.41	32.12	29.49	36.58	33.14	26.20	14.47	46.75	32.83
C6	33.47	26.06	29.60	24.49	17.08	32.21	29.33	36.94	33.27	26.29	15.99	43.54	29.01
O7	34.19	26.61	30.37	25.15	17.55	37.94	36.00	40.69	37.93	30.06	19.54	47.96	32.65
C8	35.01	27.59	32.02	26.75	17.81	40.21	41.14	42.96	40.77	35.42	19.39	48.10	25.96
F9	37.23	30.92	33.95	26.17	21.39	42.05	40.25	39.33	36.92	31.51	20.51	44.52	26.29
F10	34.37	28.34	33.49	27.89	21.61	40.81	40.70	44.50	38.13	34.70	20.97	51.19	33.31
F11	38.37	30.74	36.74	25.57	19.80	42.58	45.28	44.79	39.90	36.65	26.92	48.05	37.19
C12	32.15	26.81	30.14	28.19	17.74	31.49	27.60	35.79	33.82	31.99	13.19	54.31	30.38
N13	31.82	25.96	27.83	24.75	15.59	32.72	27.13	37.17	35.68	29.55	14.24	53.47	31.08
O14	33.99	27.53	29.31	25.68	19.60	29.31	24.16	32.04	31.21	23.66	15.21	54.57	38.28
C15	31.98	26.77	28.29	27.35	21.17	31.68	25.98	41.62	35.26	29.49	16.37	46.83	25.58
C16	32.39	26.80	30.06	28.16	21.14	29.66	24.87	42.54	33.72	29.70	16.87	42.15	22.65
O17	32.23	27.22	27.70	27.72	17.68	28.22	25.01	42.10	35.91	34.56	17.19	36.66	18.43
P18	33.68	26.69	29.23	25.97	17.25	24.56	23.07	41.24	32.67	30.86	16.83	37.69	18.93
O19	33.89	31.67	34.62	32.73	20.65	25.08	23.60	37.86	32.74	29.26	16.04	39.31	17.36
O20	33.21	31.12	34.46	31.33	18.00	25.97	23.29	41.54	30.11	34.25	17.22	35.27	22.21
O21	31.94	28.06	30.39	29.23	21.89	24.97	23.10	40.10	36.68	33.40	17.02	38.96	17.76
Mean B-factor	33.56	27.59	30.50	26.79	18.79	32.51	29.66	39.18	34.74	30.31	17.08	45.94	27.60

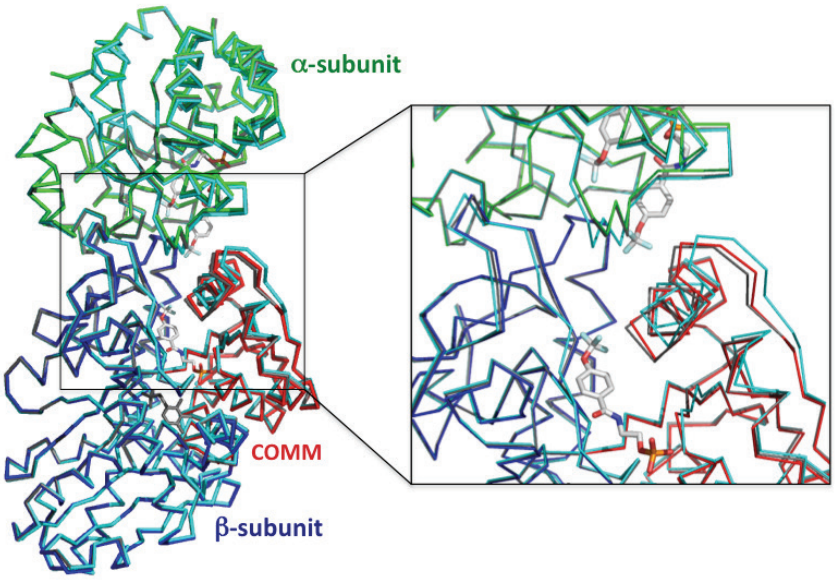


Figure S1. Structural comparison of TS with one (PDB ID: 2CLE, cyan), two (PDB ID: 2CLF, gray), and three F6 molecules (PDB ID: 4WX2), α-subunit in green, β-subunit in blue, and COMM in red).

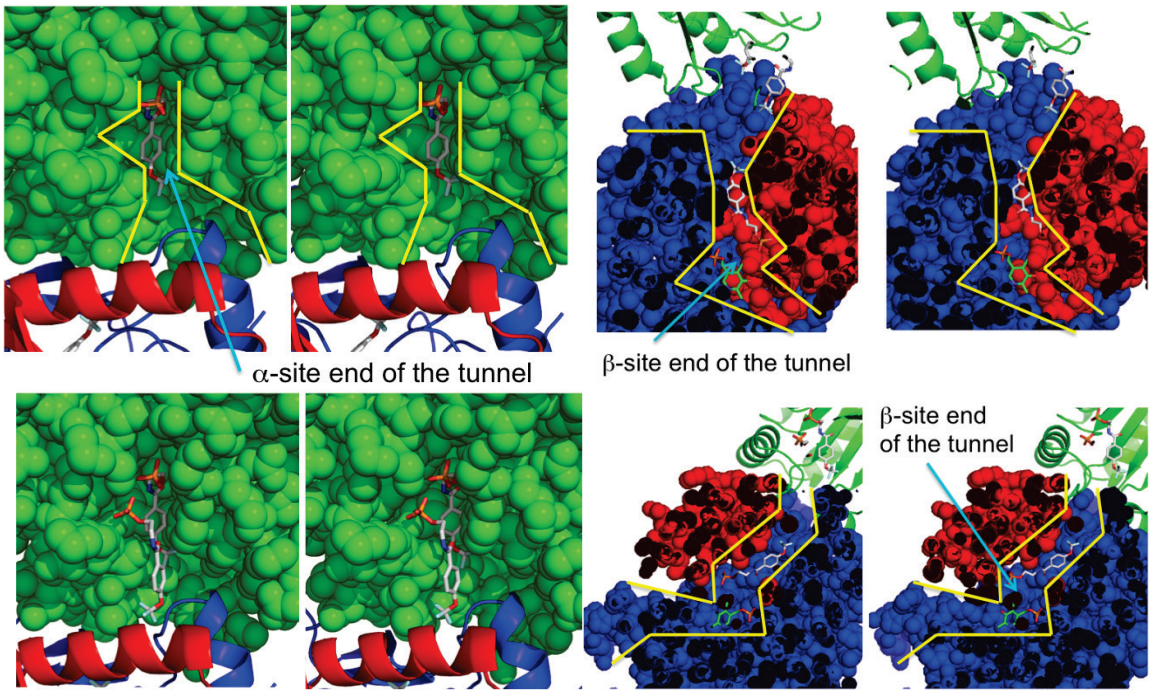


Figure S2a and S2b. Stereoviews of the tunnel connecting the α- and β-active sites and bound F6 molecules. The F6-1 molecule is bound to the β-subunit, and F6-2 and F6-3 molecules are bound to the α-subunit. The α-subunit is shown in green, the β-subunit is in blue, and COMM domain in red from the model PDB ID: 4WX2. The tunnel is highlighted by yellow lines in (a) and (b). (a), The F6-3 molecule was hidden in the highlighted α-site for a better visualization of the tunnel region.

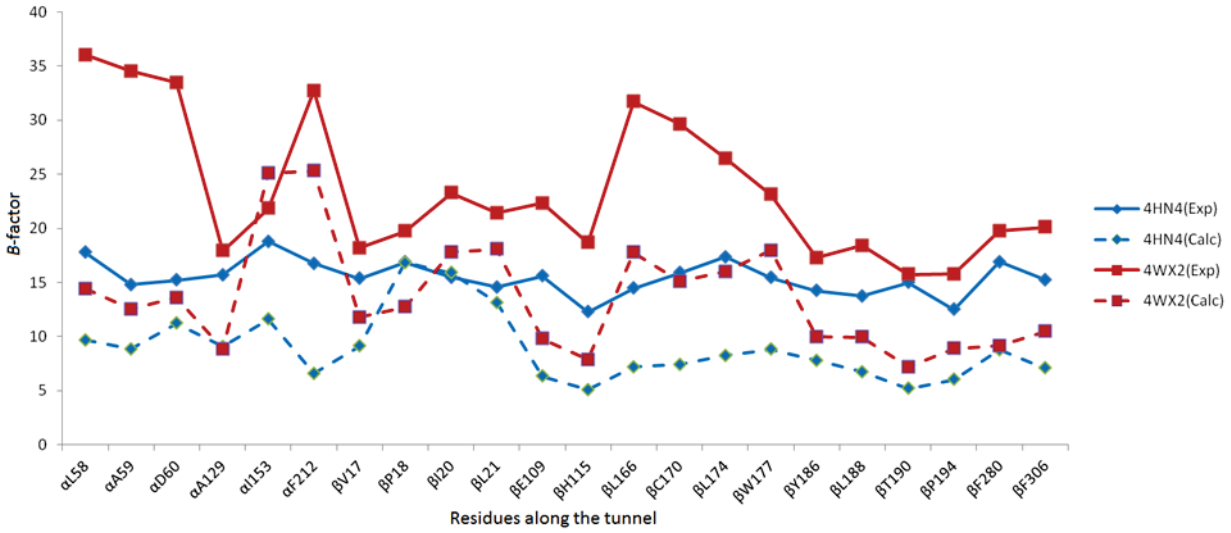
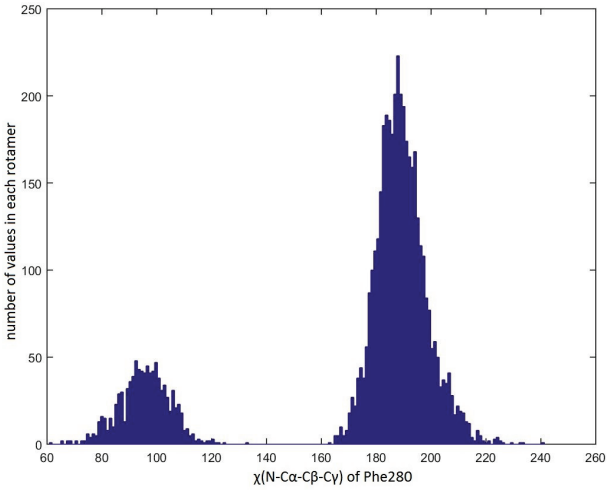


Figure S3. Comparison of both experimental (solid line) and calculated (dashed line) B factors for residues along the tunnel from 4HN4 and 4WX2. PDBID: 4WX2 has larger displacements due to the presence of F6 molecules in the β-subunit tunnel and at the αβ-subunit interface. This structure displays relatively larger B factors, especially for αLeu58, αAla59, αAsp60, αPhe212, βLeu166, βCys170, βLeu174 and βPhe306, indicating their ability to move and make room for indole to pass, which is confirmed by theoretical values calculated from mean-square fluctuations.

(a)



(b)

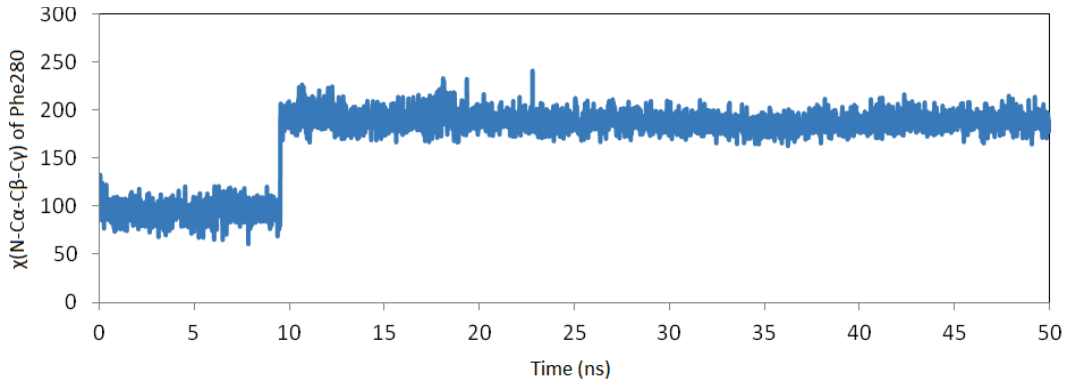


Figure S4. (a) Histogram of the main rotamers of the βPhe280 side chain from the second MD simulation. (b) βPhe280 side chain dihedral angle χ(N-C^{α}-C^{β}-C^{γ}) changes during the second MD simulation.

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