Supporting Information for

Crystallographic Snapshots of Tyrosine Phenol-lyase Show that Substrate Strain Plays a Role in C–C Bond Cleavage

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Description of the Structural Models

The final model of **F448H TPL** complex with **3-F-L-Tyr** contains 16 890 atoms, including 1 863 water molecules, four K^+ cations, four 3-fluoro-tyrosine (3-F-Tyr) quinonoid molecules in the "tense" geometry, and 24 fragments of poly(ethylene glycol) (PEG). The crystallographic asymmetric unit of F448H TPL complex with3-F-L-Tyr contains a complete tetramer of tyrosine phenol-lyase (TPL) with four crystallographically independent active sites found in the closed conformation and occupied by the "tense" 3-F-Tyr quinonoid. The electron density maps are very clear and there is no sign of domain disorder. The electron density corresponding to the ligands bound in the active sites is also well defined. The "tense" 3-F-Tyr quinonoid geometry was further confirmed by refinement with PRIMEX (Fig. S3). Several fragments of the electron density were interpreted as parts of poly(ethylene glycol) monomethyl ether (PEG MME) chains.[1] There are two heptameric fragments (seven $–CH_2CH_2O–$ mers) found between the two catalytic dimers, as in the crystal structures of *Citrobacter freundii* TPL quinonoid complexes formed with L-Ala and L-Met.[2] All modeled PEG fragments in F448H TPL–F-Tyr are listed in Table S1. Most of them are bound in the equivalent sites of different protein subunits. Residues Ile43(A), Glu75(A), His79(A), Cys179(A), Arg198(A), Arg397(A), Asp422(A), Arg452(A), Ile43(B), Glu75(B), His79(B), Cys179(B), Arg198(B), Val211(B), Asp422(B), Glu23(C), Ile43(C), Glu75(C), His79(C), Arg82(C), Lys132(C), Cys179(C), Val211(C), Asp422(C), Arg452(C), Ile43(D), His79(D), Cys179(D), Arg198(D), Val211(D), Asp422(D), and Arg452(D) were modeled in two alternate conformations with 0.5 occupancy.

The final model of **Y71F TPL** complex with**3-F-L-Tyr** is composed of 8 131 atoms, including 745 water molecules, 2 K^+ cations, 1.33 3-F-Tyr quinonoid molecules in the "relaxed" conformation, 0.67 3-F-L-Tyr quinonoid molecules in the "tense" conformation and 2 fragments of PEG. There is a catalytic dimer in the crystallographic asymmetric unit. Subunit B of the dimer is found in the open conformation with a "relaxed" quinonoid molecule bound in its active site. A major proportion of the active site A is occupied by the quinonoid molecule in the "tense" state with occupancy estimated at 0.67. As in the structure of TPL methionine quinonoid,[2] the residual electron density suggests that a minor population of subunits A is in the open conformation. Due to the unconnected peaks in the electron density maps and the low occupancy, it was not possible to model this open conformation. The electron density in the active site of subunit A indicates that apart from the "tense" quinonoid there is an additional chemical species (Fig. S2). The residual electron density is best fitted by a "relaxed" quinonoid modeled with the estimated occupancy of 0.33 in the same geometry as in the "open" active site B. The quinonoid molecule with such "relaxed" geometry cannot exist in the closed active site due to a significant steric clash between the phenol moiety and the side chains of Phe448 and Phe449 with distances between non-hydrogen atoms as short as 1.9 Å. Two PEG fragments (one tetrameric and one dimeric) were modeled in the groove between the two catalytic dimers, *i.e.* in the PEG binding site which is also found in other TPL structures.[2] Residues Ser17(A), Asp22(A), His79(A), Arg198(A), Arg351(A), Asp422(A), His79(B), and Cys179(B) were modeled in two alternate conformations with occupancies of 0.5.

The final model of **TPL–L-Ala–PNO** comprises 8 174 atoms, including 792 water molecules, 2 K^+ cations, 1 PO_4^{3-}, 1.5 PLP, 0.5 alanine quinonoid intermediate, 1 PEG fragment, and 3 pyridine *N*-oxide (PNO) molecules. The crystallographic asymmetric unit contains the catalytic dimer with one subunit in the open and the second in the closed conformation. The electron density is very clear, with no discrete disorder in domain positions. However, while the open active site (B) is occupied only by the internal aldimine, the closed active site (A) is occupied by PNO molecule and a mixture of different ligands (Fig. S12). Namely, half of the closed active sites are occupied by the Ala quinonoid intermediate. The other half is occupied by the internal aldimine, the water molecule and a phosphate anion (from the purification buffer) bound in the favorable phosphate-binding site (formed only in the closed conformation) by the side chains of Arg404(A), Arg381(A), Arg217(A), Asn185(A) and Thr49(A). This phosphate-binding site is equivalent to the second sulfate-binding site in the structural form I of *Escherichia coli* apo-tryptophan indole-lyase.[3] There is no PNO molecule in the open active site (B). Instead, it is found near the entrance of the active-site cleft, next to Asp348(B) [Asp348(B) OD2···PNO N 3.2 Å]. This PNO molecule is H-bonded to the water molecule which is fixed by H-bonds formed with the amide and carbonyl groups of Glu401(B), and the carboxyl group of Asp348(B). The PNO molecule located at this site is also surrounded by the hydrophobic side chains of Ala148(B), Val337(B) and Leu400(B). Another PNO molecule is situated next to the N-terminal arms. It is stacked between the indole rings of two Trp61 residues, each from a different catalytic dimer, and H-bonded to the side chain of Ser12(A) and a water molecule which forms H-bonds with the carbonyl group of Met65(B) and the side chain of Lys11(B) from the second catalytic dimer. The heptameric PEG fragment was found in the cleft between the two catalytic dimers. Met1(A) and Met1(B) could not be modeled in the electron density maps. Residues Ser17(A), His79(A), Asp147(A), Lys257(A), Glu273(A), Lys328(A), Asp422(A), Met66(B), and His430(B) were modeled in two alternate conformations with occupancies of 0.5.

Table S1. PEG Fragments Modeled in the Structure of F448H TPL–3-F-L-Tyr

PDB component identifier	No. of $–CH_2CH_2O–$ mers	Location (next to listed residues)
PGE	3	Lys170(A), Glu169(A), Lys165(A)
PGE	3	Lys226(A)
PGE	3	Glu313(A)
PG4	4	Lys444(A)
PGE	3	Lys444(A)
P33	7	between subunits A and D
PG4	4	Lys170(B), Glu169(B), Lys165(B)
PGE	3	Lys226(B)
PGE	3	Glu313(B)
PG4	4	Lys444(B)
EDO	1	Lys444(B)
PGE	3	Met66(B) and Met66(D)
P33	7	between subunits B and C
PGE	3	Lys170(C), Glu169(C), Lys165(C)
PGE	3	Lys226(C)
1PE	5	Glu313(C)
PGE	3	Lys444(C)
PEG	2	Lys444(C)
PGE	3	Lys170(D), Glu169(D), Lys165(D)
PGE	3	Lys226(D)
PG4	4	Glu313(D)
PGE	3	Lys444(D)
PEG	2	Lys112(D)
PG4	4	Lys444(D)

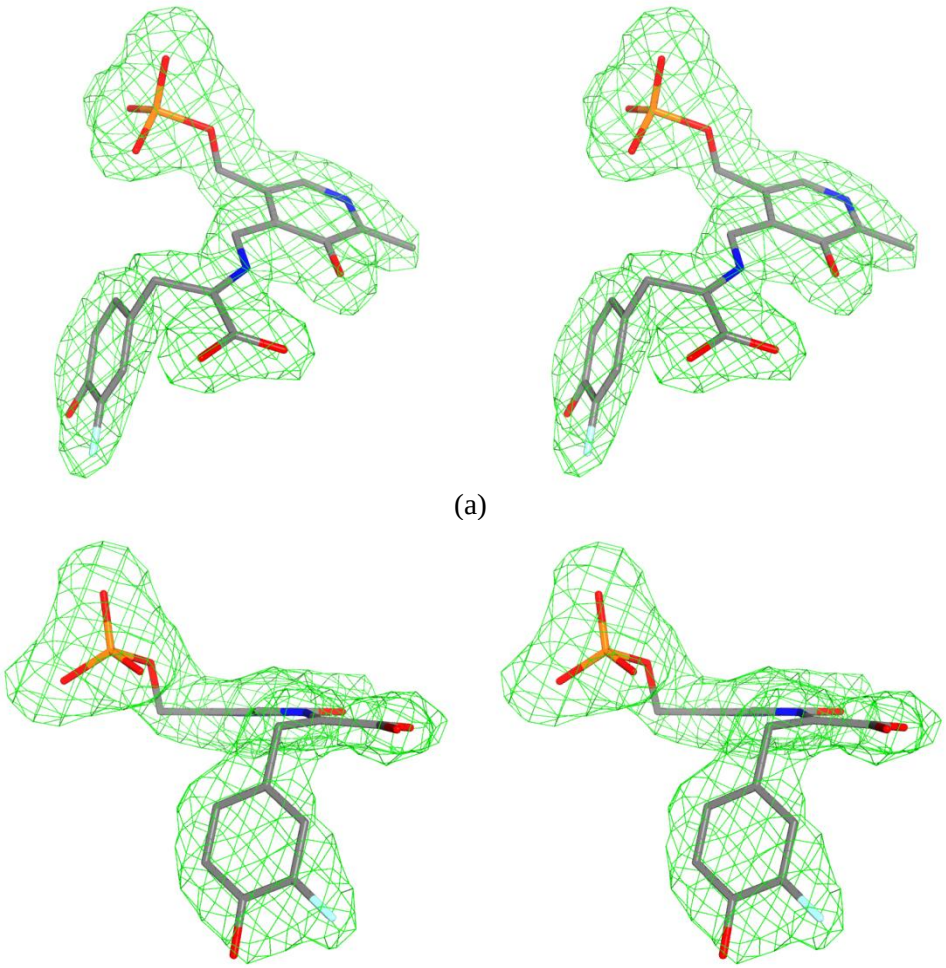
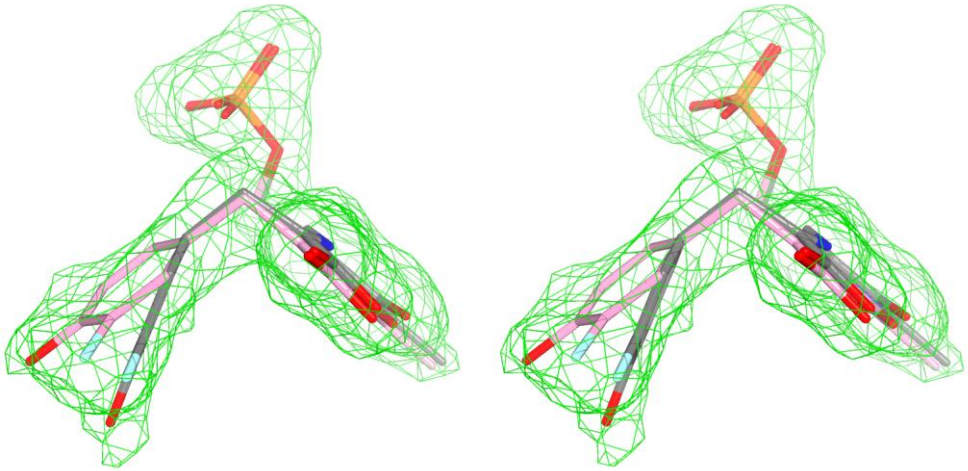
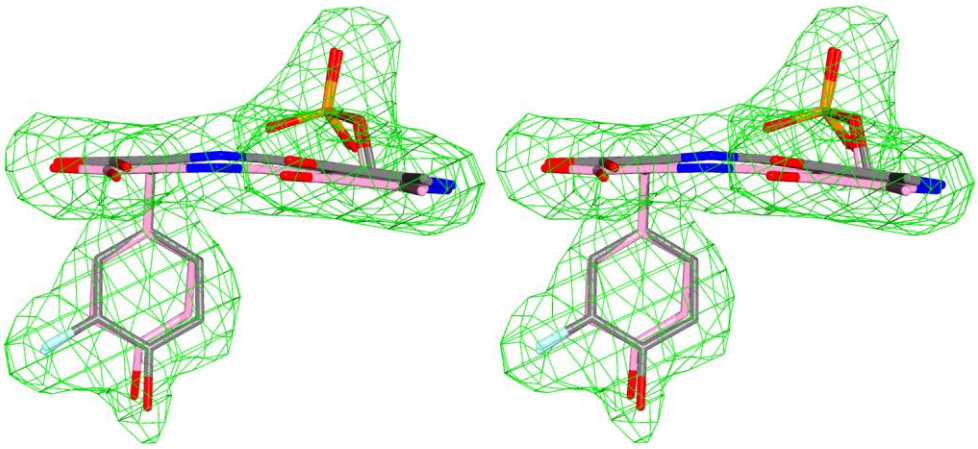


Figure S1. 3-F-Tyr quinonoid molecule in the open active site of Y71F TPL. Two different stereo views, (a) and (b). The "relaxed" quinonoid molecule is superimposed with the corresponding σ_A-weighted $|F_o|-|F_c|$ omit electron density maps (*green*) contoured at 3.0σ.

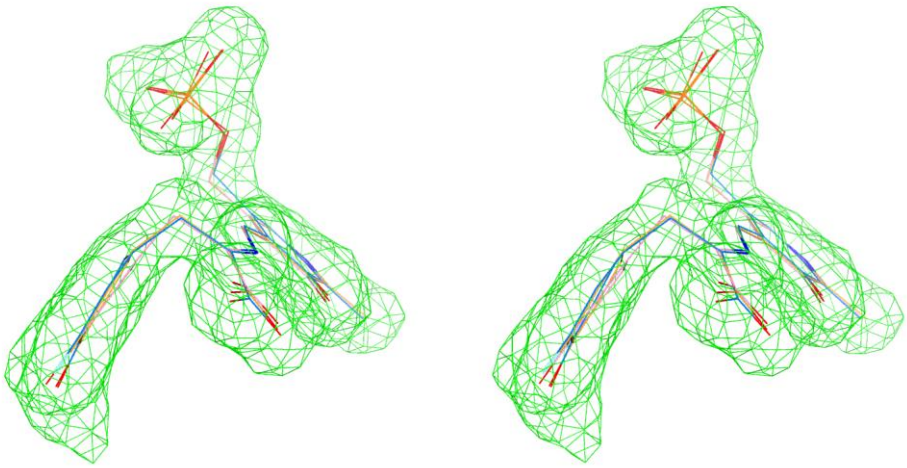


(a)

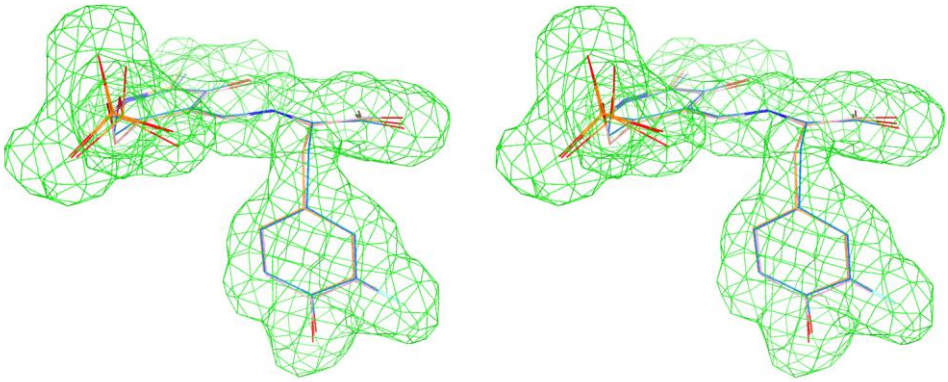


(b)

Figure S2. Quinonoid molecules modeled in the disordered active site of Y71F TPL. Two different stereo views, (a) and (b), with the "relaxed" (*pink*) and "tense" (*grey*) quinonoid molecules superimposed with the corresponding σ_A-weighted $|F_o|–|F_c|$ omit electron density omit maps (*green*) contoured at 3.0σ.

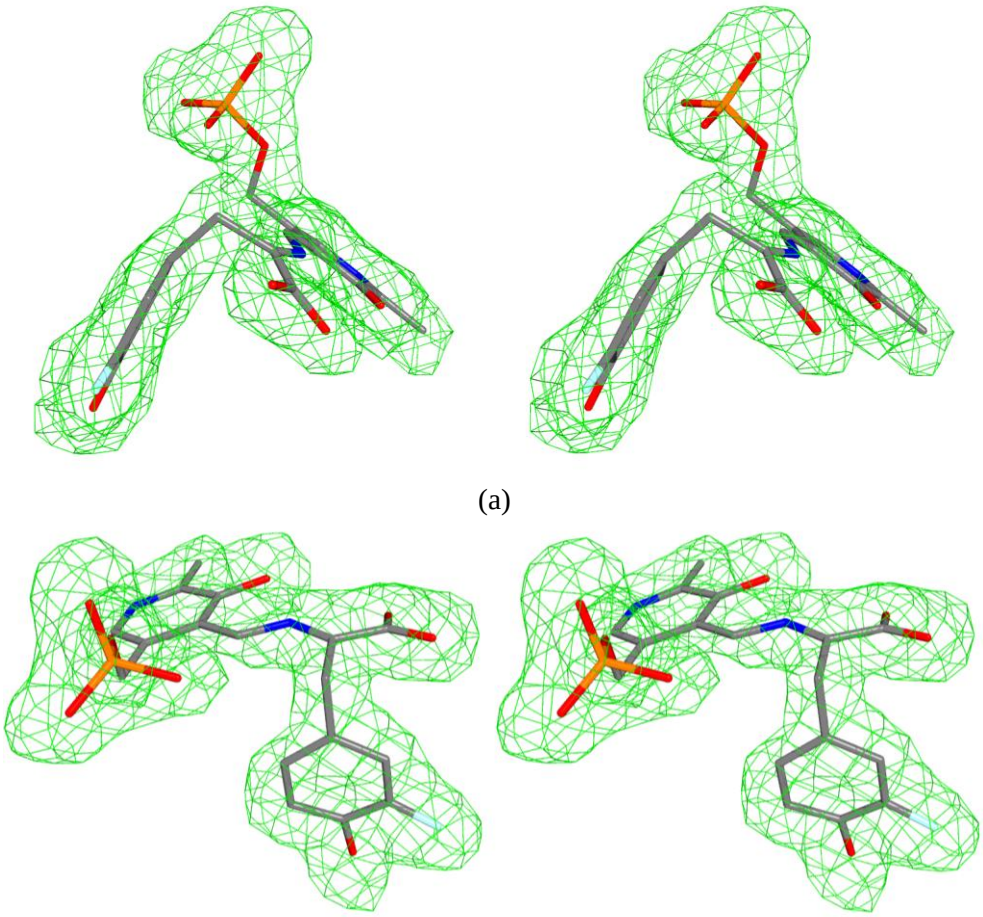


(a)



(b)

Figure S3. Ligands with different geometries can be fitted in the active sites of the F448H TPL–3-F-L-Tyr. Two different stereo views of the quinonoid intermediates refined in REFMAC with the standard (*pink*) and "relaxed" (*orange*) geometrical restraints superposed with the quinonoid molecule refined in PRIMEX (*blue*; hydrogen atoms omitted for clarity). The σ_A-weighted $|F_o|-|F_c|$ omit electron density map (*green*) is contoured at 3.0σ. Figure corresponds to the active site B; electron density maps in the other three active sites are very similar (*cf.* Figs. S4–S7) as well as the models obtained by the different refinement strategies.



(a)

(b)

Figure S4. The "tense" 3-F-Tyr quinonoid molecule in the active site A of F448H TPL. Two different stereo views with the quinonoid molecule superimposed with the corresponding σ_A-weighted $|F_o|-|F_c|$ omit electron density maps (*green*) contoured at 3.0σ.

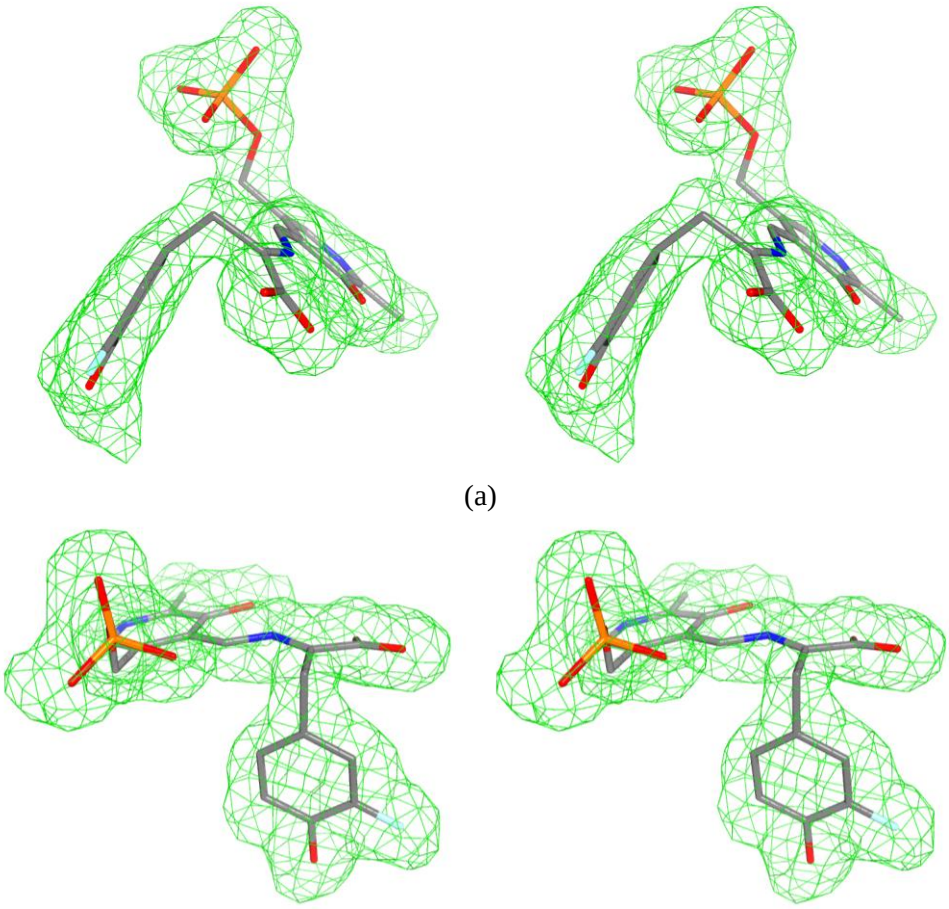
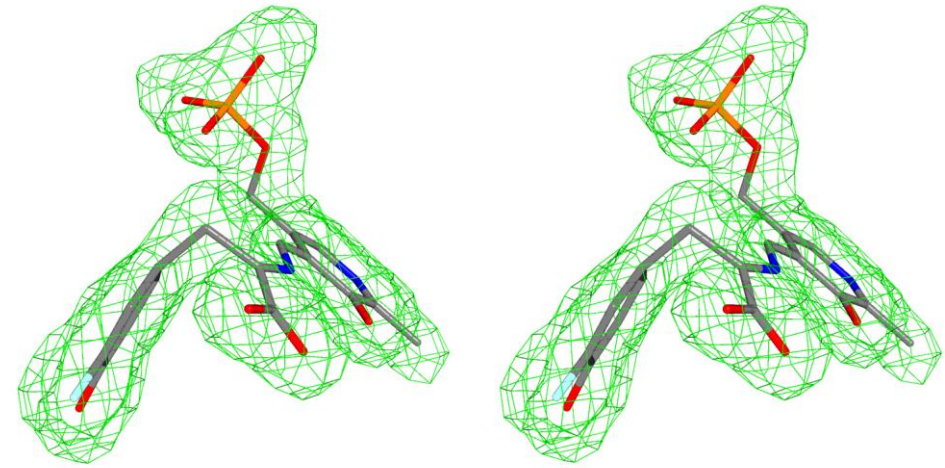


Figure S5. The "tense" 3-F-Tyr quinonoid molecule in the active site B of F448H TPL. Two different stereo views with the quinonoid molecule superimposed with the corresponding σ_A-weighted $|F_o|-|F_c|$ omit electron density maps (*green*) contoured at 3.0σ.



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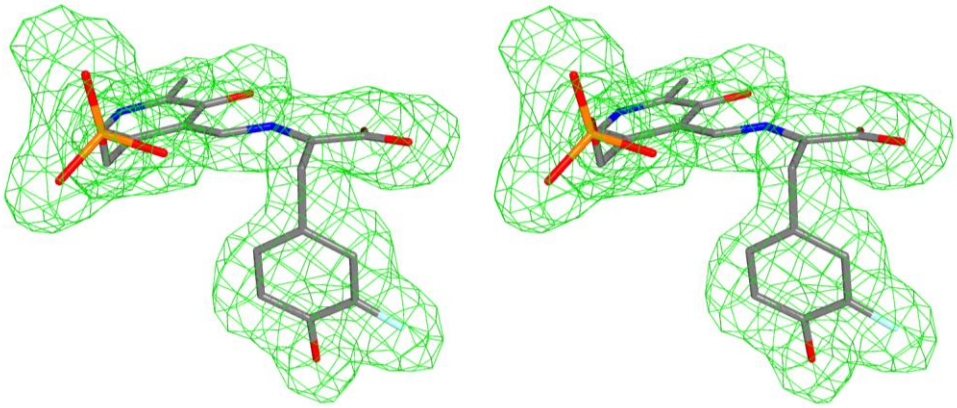
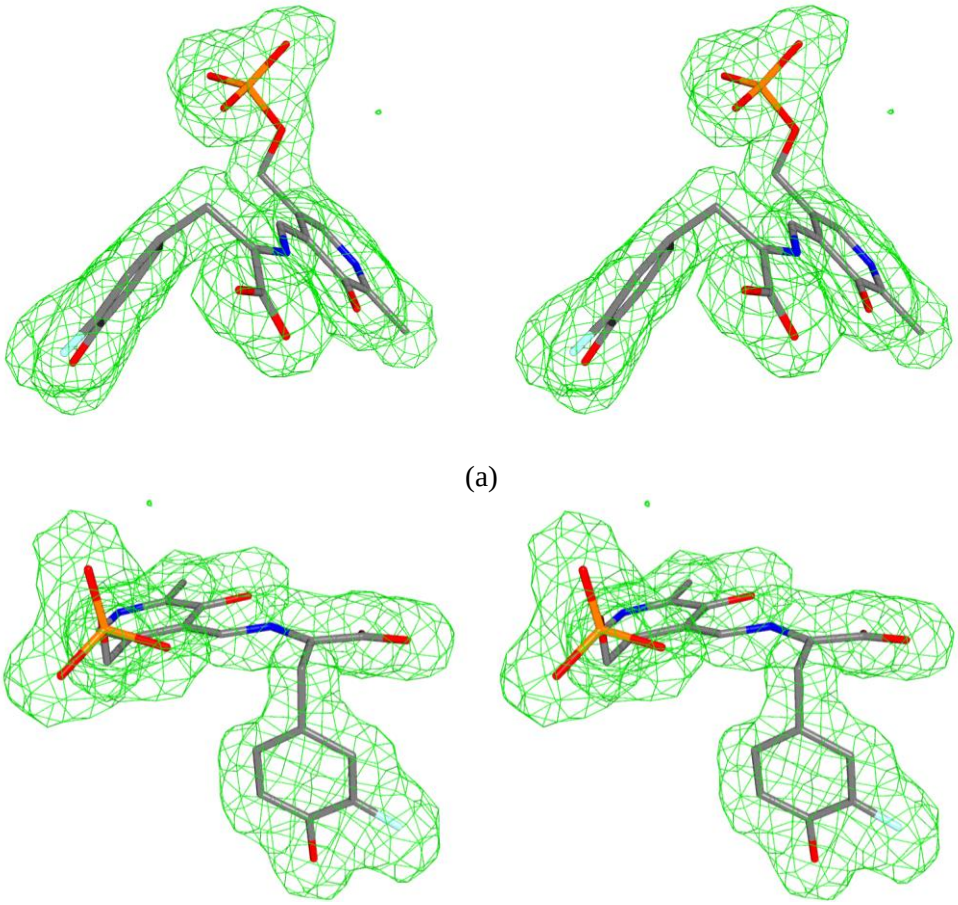


(b)

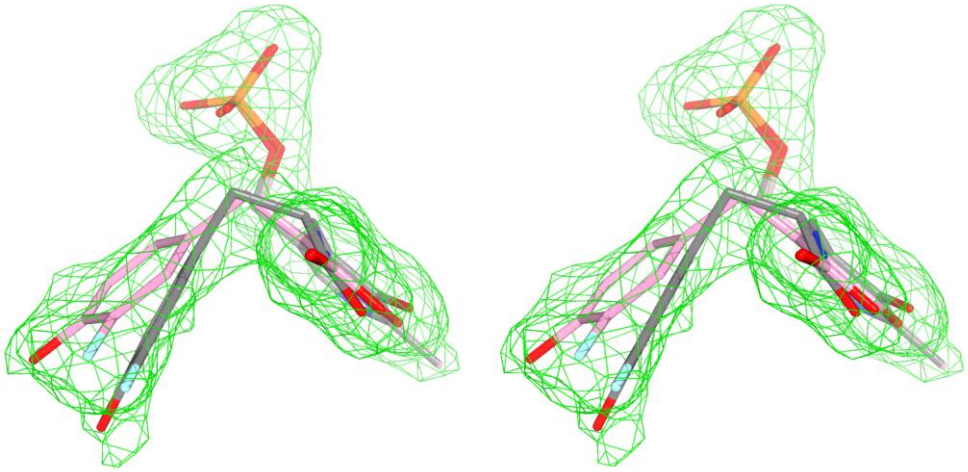
Figure S6. The "tense" 3-F-Tyr quinonoid molecule in the active site C of F448H TPL. Two different stereo views with the quinonoid molecule superimposed with the corresponding σ_A-weighted $|F_o|-|F_c|$ omit electron density maps (*green*) contoured at 3.0σs.



(a)

(b)

Figure S7. The "tense" 3-F-Tyr quinonoid molecule in the active site D of F448H TPL. Two different stereo views with the quinonoid molecule superimposed with the corresponding σ_A-weighted $|F_o|-|F_c|$ omit electron density omit maps (*green*) contoured at 3.0σ.



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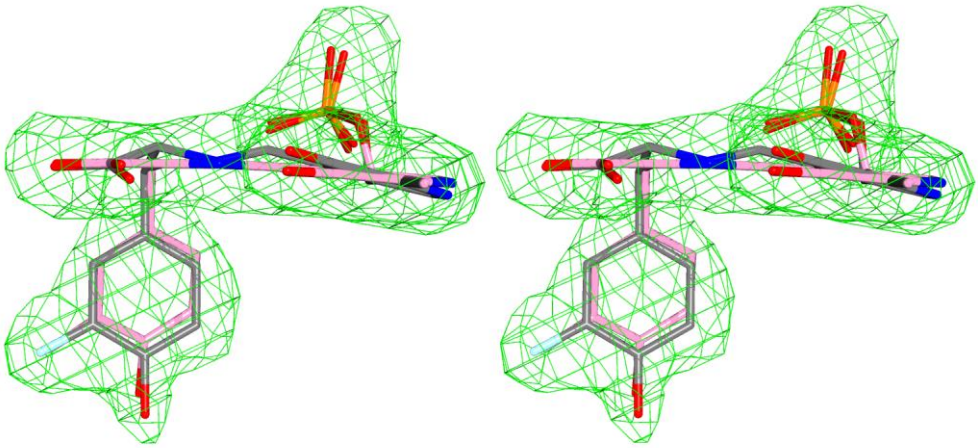
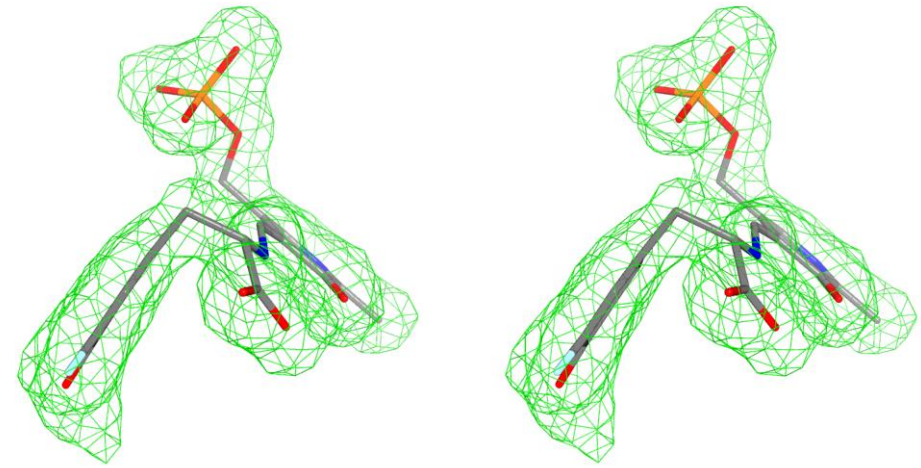


(b)

Figure S8. External aldimine modeled in the disordered active site of Y71F TPL. Two different stereo views, (a) and (b), with the "relaxed" quinonoid (*pink*) and the external aldimine (*grey*) molecules superimposed with the corresponding σ_A-weighted $|F_o|-|F_c|$ omit electron density maps (*green*) contoured at 3.0σ.



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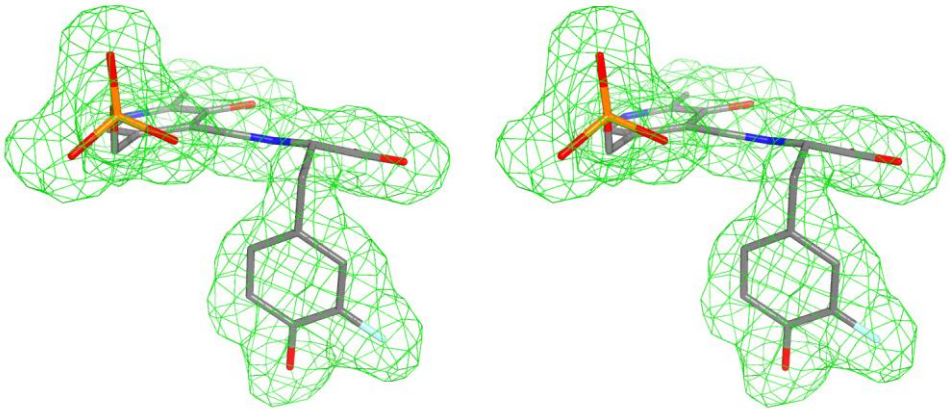


(b)

Figure S9. The external aldimine molecule modeled in the active site B of F448H TPL. Two different stereo views, (a) and (b), are shown. The external aldimine molecule is superimposed with the σ_A-weighted $|F_o|-|F_c|$ omit electron density maps (*green*) contoured at 3.0σ . Fits of the external aldimine model to the electron density in the other three active sites of F448H TPL are very similar (*cf.* Fig. S11).

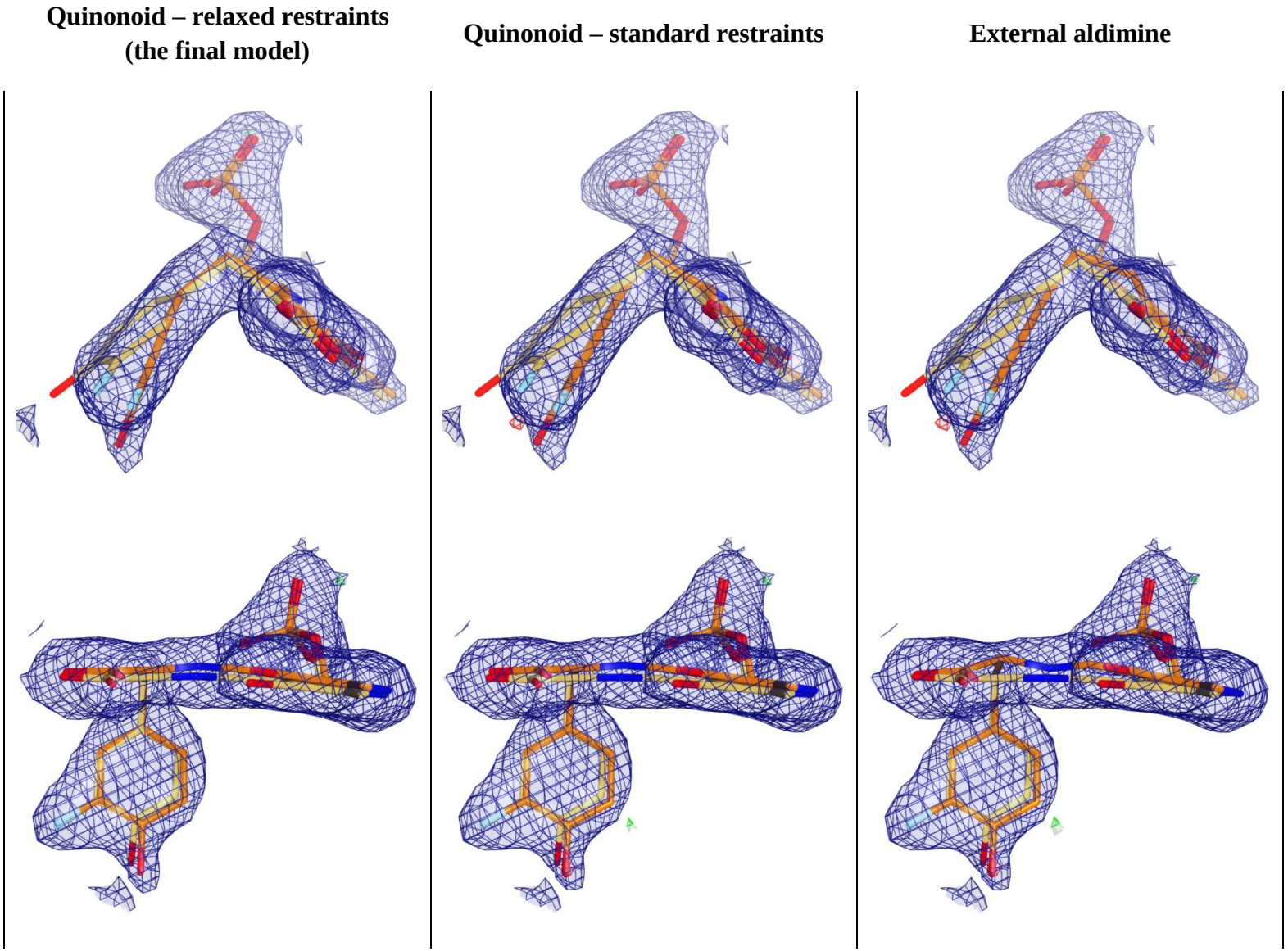
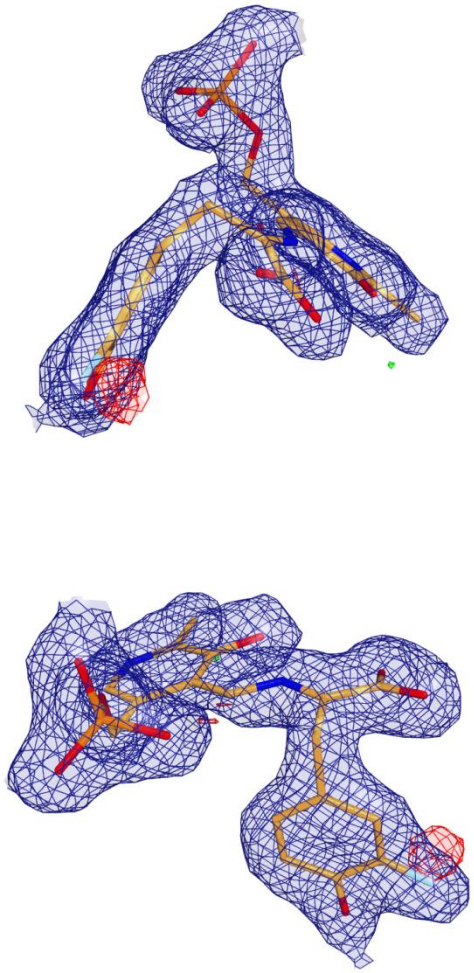
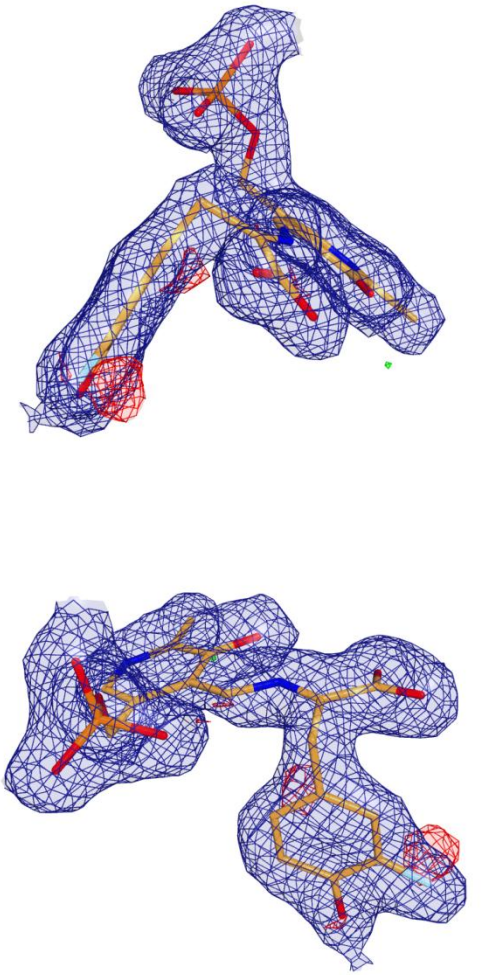
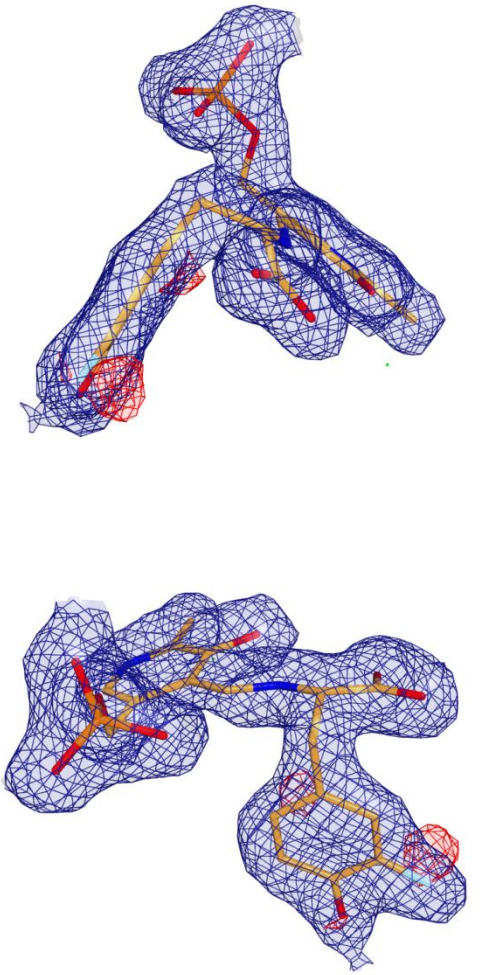
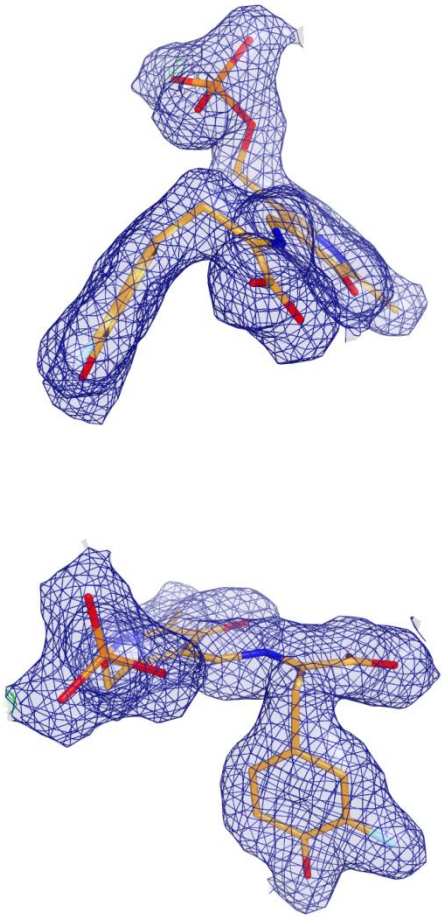
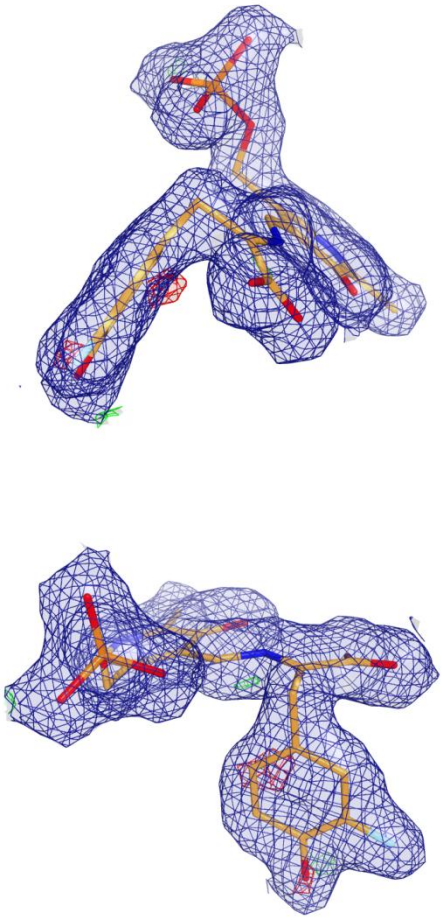
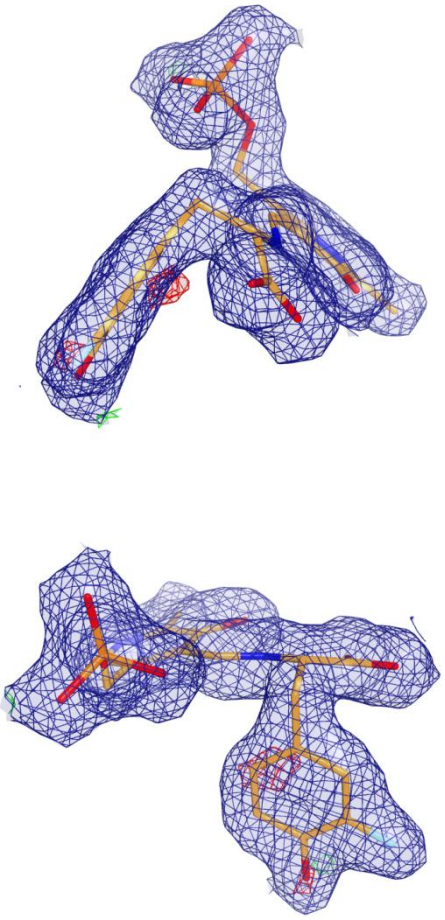


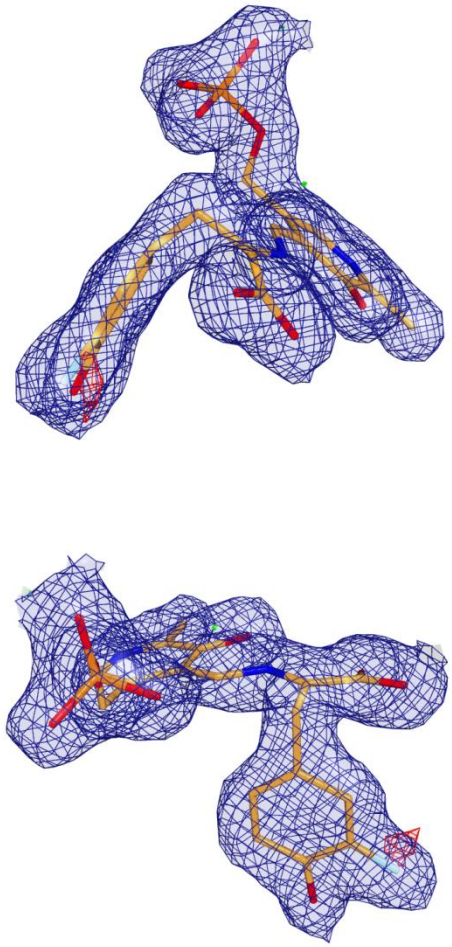
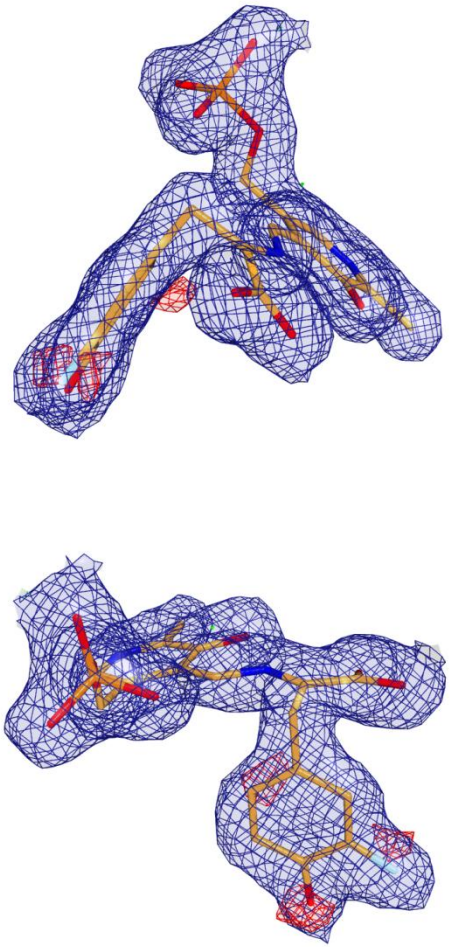
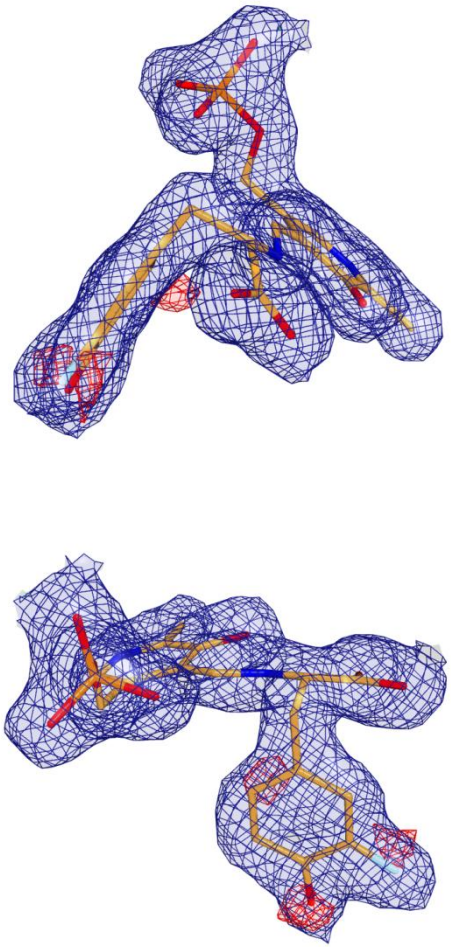
Figure S10. Different ligand models (*orange*) refined in the disordered active site of Y71F TPL. Each model is shown in two different views. The corresponding σ_A-weighted $2|F_o|–|F_c|$ electron density map (*blue*) is contoured at 1.0σ level and the σ_A-weighted $|F_o|–|F_c|$ electron density map is contoured at –3.0σ (*red*) and 3.0σ (*green*). The "relaxed" quinonoid (0.33 occupancy) refined with standard restraints is shown in *yellow*.

Quinonoid – relaxed restraints (the final model)	Quinonoid – standard restraints	External aldimine
		

(a)

Quinonoid – relaxed restraints (the final model)	Quinonoid – standard restraints	External aldimine
		

(b)

Quinonoid – relaxed restraints (the final model)	Quinonoid – standard restraints	External aldimine
		

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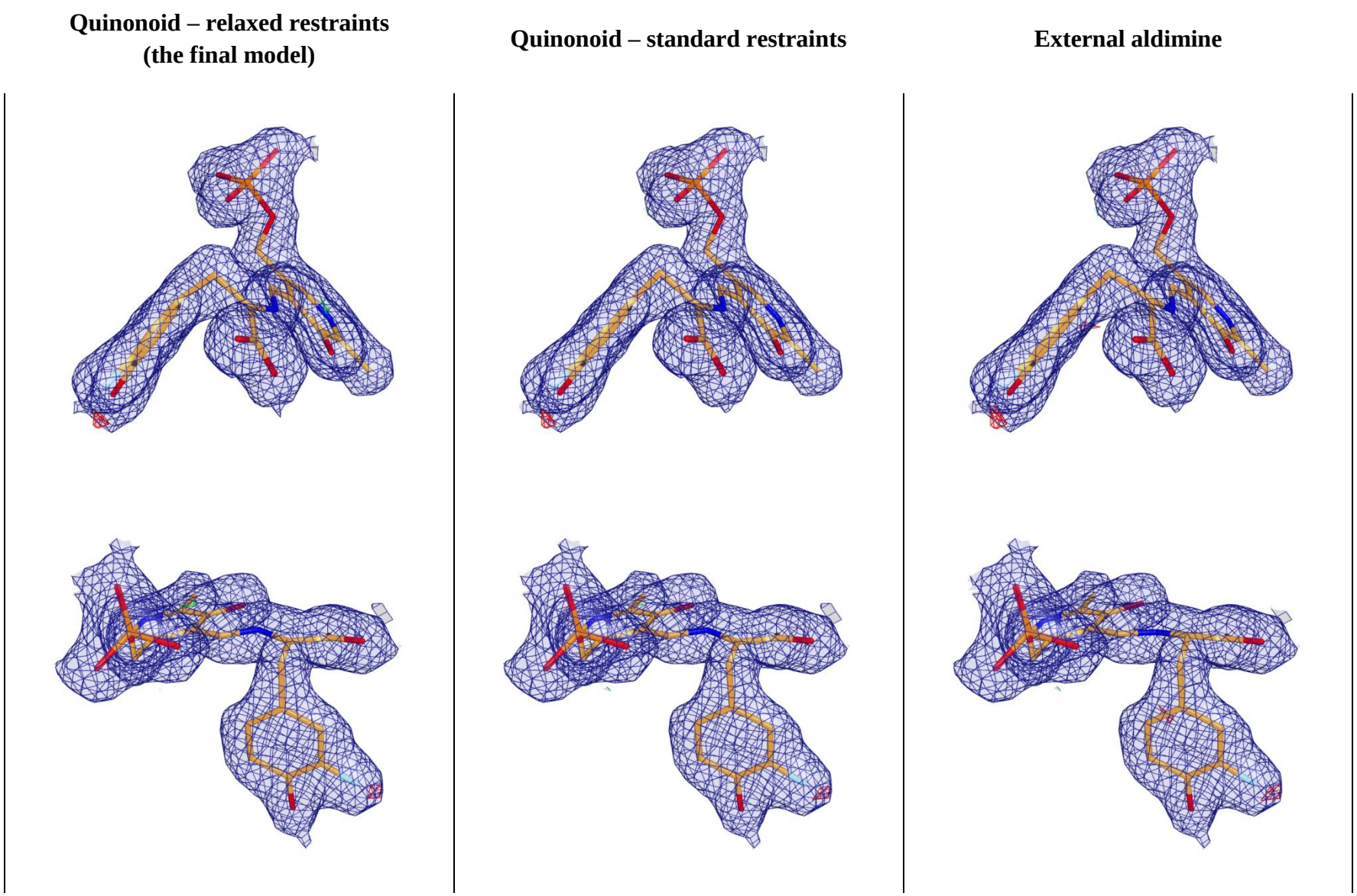
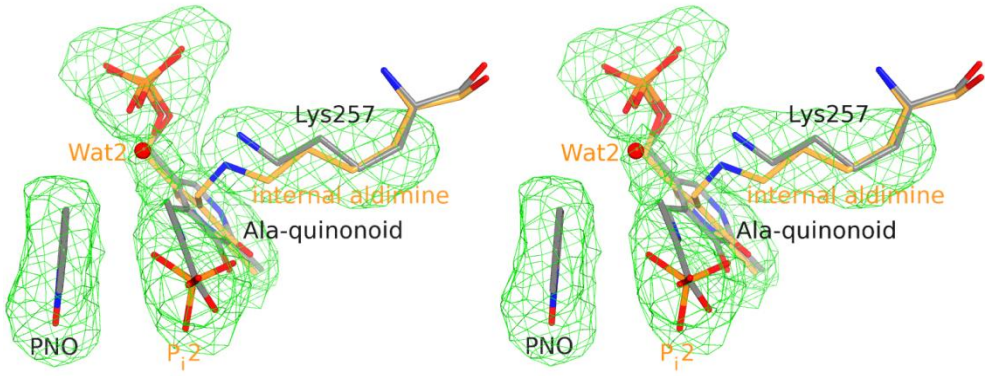
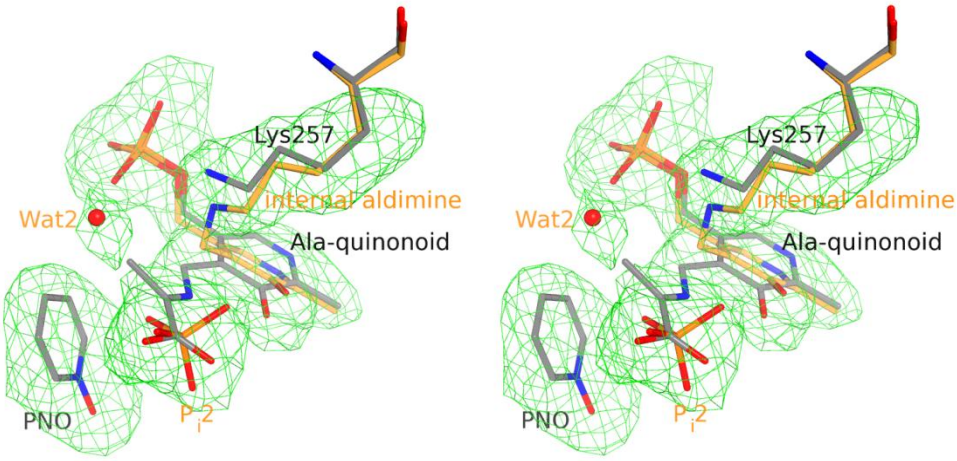


Figure S11. Different ligand models (*orange*) refined in the four crystallographically independent active sites of F448H TPL, (*a*)–(*d*). Each model is shown in two different views. The corresponding σ_A-weighted $2|F_o|–|F_c|$ electron density map (*blue*) is contoured at 1.0σ level and the σ_A-weighted $|F_o|–|F_c|$ electron density map is contoured at –3.0σ (*red*) and 3.0σ (*green*) levels.



(a)



(b)

Figure S12. Ligands in the closed active site of the alanine quinonoid intermediate in complex with pyridine *N*-oxide (PNO). Two different stereo views, (a) and (b), with the ligand molecules superimposed with the corresponding σ_A-weighted $|F_o|–|F_c|$ omit electron density map (*green*) contoured at 2.0σ. The internal aldimine, alanine quinonoid intermediate (Ala-quinonoid), phosphate anion (P_i2) and water molecule Wat2 are modeled with 0.5 occupancy.

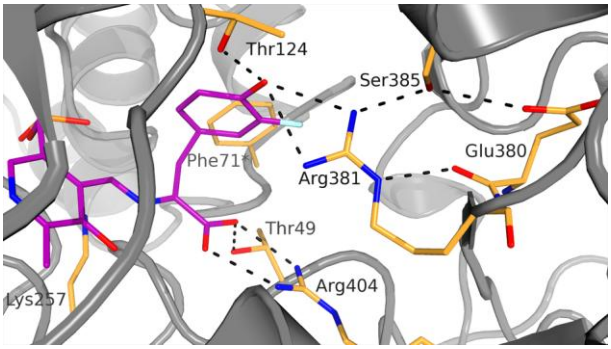


Figure S13. Interaction of the Arg381–Ser385–Glu380 triad with the "tense" quinonoid (*purple*) in the closed conformation of Y71F TPL. Hydrogen bonds are shown by *dashed lines*.

References

(1) Hašek, J. *Z. Kristallogr. Suppl.* **2006**, *23*, 613–618.
(2) Milić, D.; Demidkina, T. V.; Faleev, N. G.; Matković-Čalogović, D.; Antson, A. A. *J. Biol. Chem.* **2008**, *283*, 29206–29214.
(3) Ku, S.-Y.; Yip, P.; Howell, P. L. *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **2006**, *62*, 814–823.