SUPPLEMENTAL MATERIAL

Table S1. Amino acids 90-104 are strictly conserved in Class I EPSP synthases.

EPSPS	Amino Acid Residue
Escherichia coli	^{90}**LFLGN AGTAM RPLAA**^{104}
Klebsiella pneumoniae	^{90}**LFLGN AGTAM RPLAA**^{104}
Salmonella typhi	^{90}**LFLGN AGTAM RPLAA**^{104}
Zea mays	^{95}**LFLGN AGTAM RPLTA**^{109}
Petunia hybrida	^{167}**LFLGN AGTAM RPLTA**^{181}

TABLE S2. Changes in backbone torsion angles of residues 96 to 98 as a result of Thr^{97}/Pro^{101} mutations.

	WT EPSPS			T97I EPSPS			TIPS EPSPS		
S3P + Glyphosate bound	Gly^{96}	Thr^{97}	Ala^{98}	Gly^{96}	Thr^{97}	Ala^{98}	Gly^{96}	Thr^{97}	Ala^{98}
Torsion Angle[a]									
Φ	-63°	-72°	-104°	-61°	-78°	-106°	-58°	-62°	-67°
ψ	-34°	-5.6°	-50°	-39°	5.0°	-50°	-50°	-36°	-48°
ω	-178°	170°	-178°	-178°	171°	-178°	-178°	178°	180°
Planarity	0.32°	-0.03°	0.84°	0.1°	0.23°	-0.3°	-0.5°	-1.7°	-0.27°
S3P bound	Gly^{96}	Thr^{97}	Ala^{98}	Gly^{96}	Thr^{97}	Ala^{98}	Gly^{96}	Thr^{97}	Ala^{98}
Torsion Angle									
Φ	-59°	-72°	-106°	-61°	-76°	-107°	-57°	-61°	-61°
ψ	-38°	-4.3°	-47°	-40°	3.4°	-50°	-56°	-41°	-47°
ω	-179°	172°	-178°	-178°	173°	-179°	-180°	179°	179°
Planarity	-0.14°	1.65°	-0.25°	-0.6°	-0.1°	-0.83°	1.0°	-0.88°	0.4°

[a] The torsion angles were calculated with Moleman2 (1), with Φ = C(i-1) - N(i) - CA(i) - C(i), = ψ = N(i) - CA(i) - C(i) - N(i+1), ω = CA(i) - C(i) - N(i+1) - CA (i+1), and planarity = C(i) - CA(i) - N(i+1) - O(i)

(1) Kleywegt, G. J. (2001) *International Tables for Crystallography,* Kluwer Academic Publishers, **Volume F**, 497-506, 526-528.

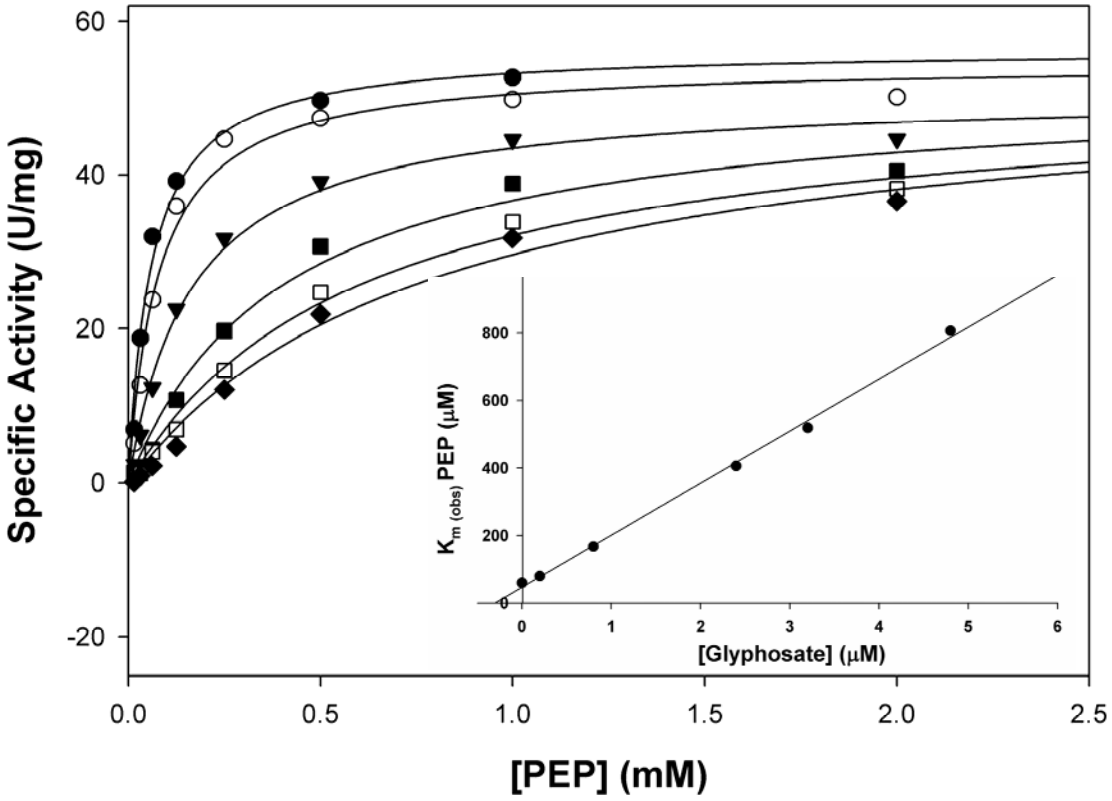


FIGURE S1. **Inhibition of wild-type EPSPS by glyphosate: determination of K_i.**
Substrate saturation in the presence of increasing concentrations of glyphosate: 0 μM (●), 0.2 μM (○), 0.8 μM (▼), 2.4μM (■), 3.2 μM (□), and 4.8 μM (◆). S3P concentration was 1 mM. Enzyme concentration was 1.4 nM. Data were fit to the Michaelis-Menten equation.
Inset: Replot of the observed K_m values as a function of glyphosate concentration. Data were fit to equation (1) yielding K_i of 0.3 ± 0.07 μM.

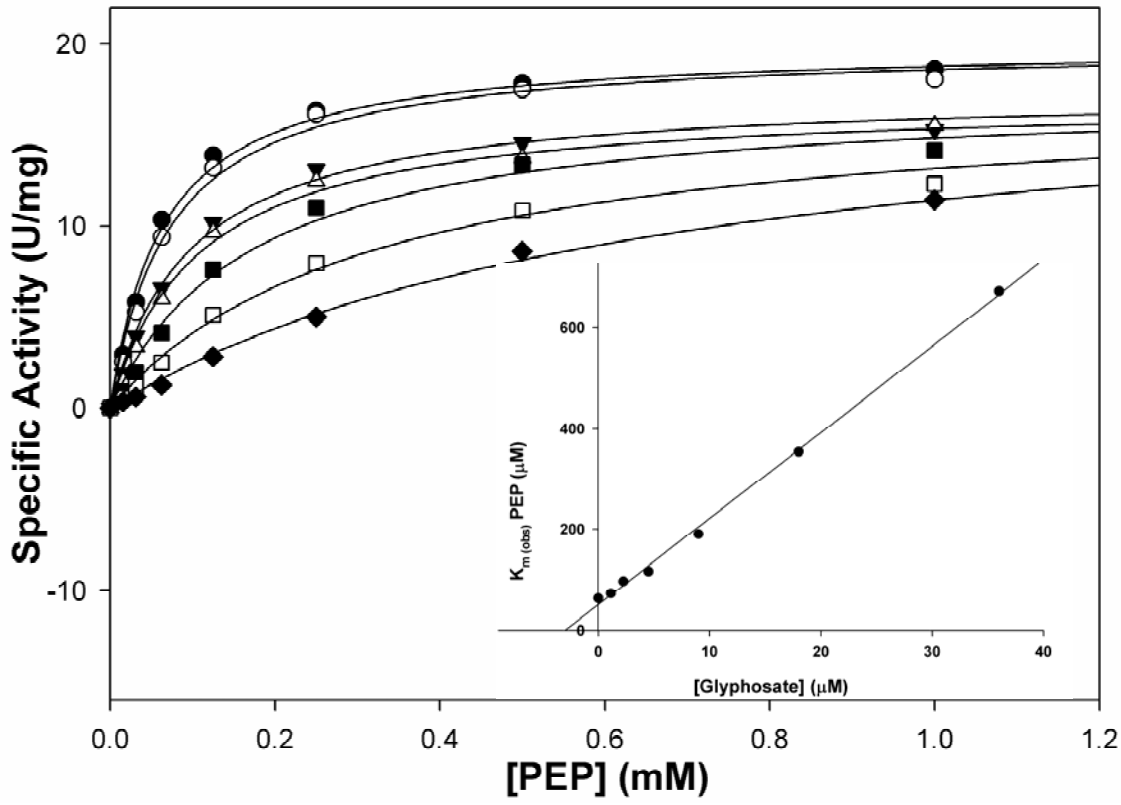


FIGURE S2. **Inhibition of P101S EPSPS by glyphosate: determination of K_i.**
Substrate saturation in the presence of increasing concentrations of glyphosate: 0 μM (●), 1.1 μM (○), 2.3 μM (▼), 4.5 μM (△), 9.0 μM (■), 18.0 μM (□), and 36.0 μM (◆) S3P concentration was 1 mM. Enzyme concentration was 2.9 nM. Data were fit to the Michaelis-Menten equation.
Inset: Replot of the observed K_m values as a function of glyphosate concentration. Data were fit to equation (1) yielding K_i of 3.0 ± 0.3 μM.

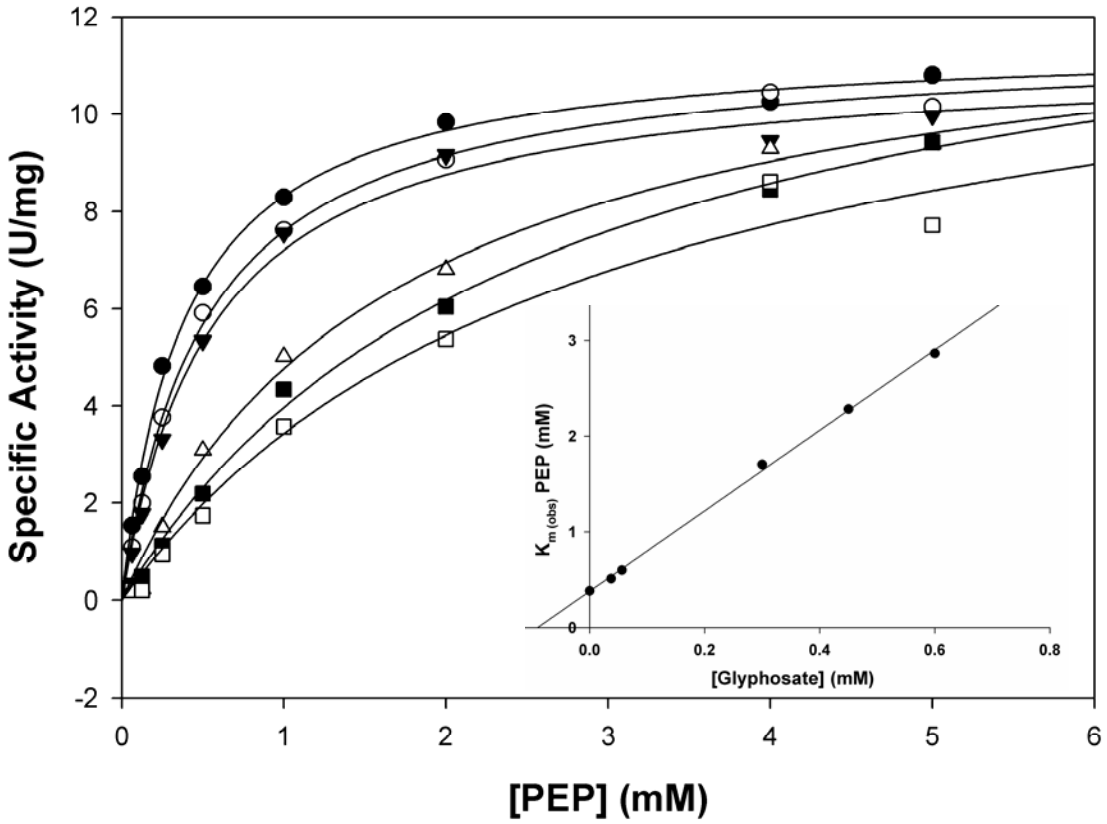


FIGURE S3. **Inhibition of T97I EPSPS by glyphosate: determination of K_i.**
Substrate saturation in the presence of increasing concentrations of glyphosate: 0 µM (●), 0.038 mM (○), 0.056 mM (▼), 0.30 mM (△), 0.45 mM (■), and 0.60 mM (□). S3P concentration was 1 mM. Enzyme concentration was 5.1 nM. Data were fit to the Michaelis-Menten equation.
Inset: Replot of the observed K_m values as a function of glyphosate concentration. Data were fit to equation (1) yielding K_i of 90 ± 6 µM

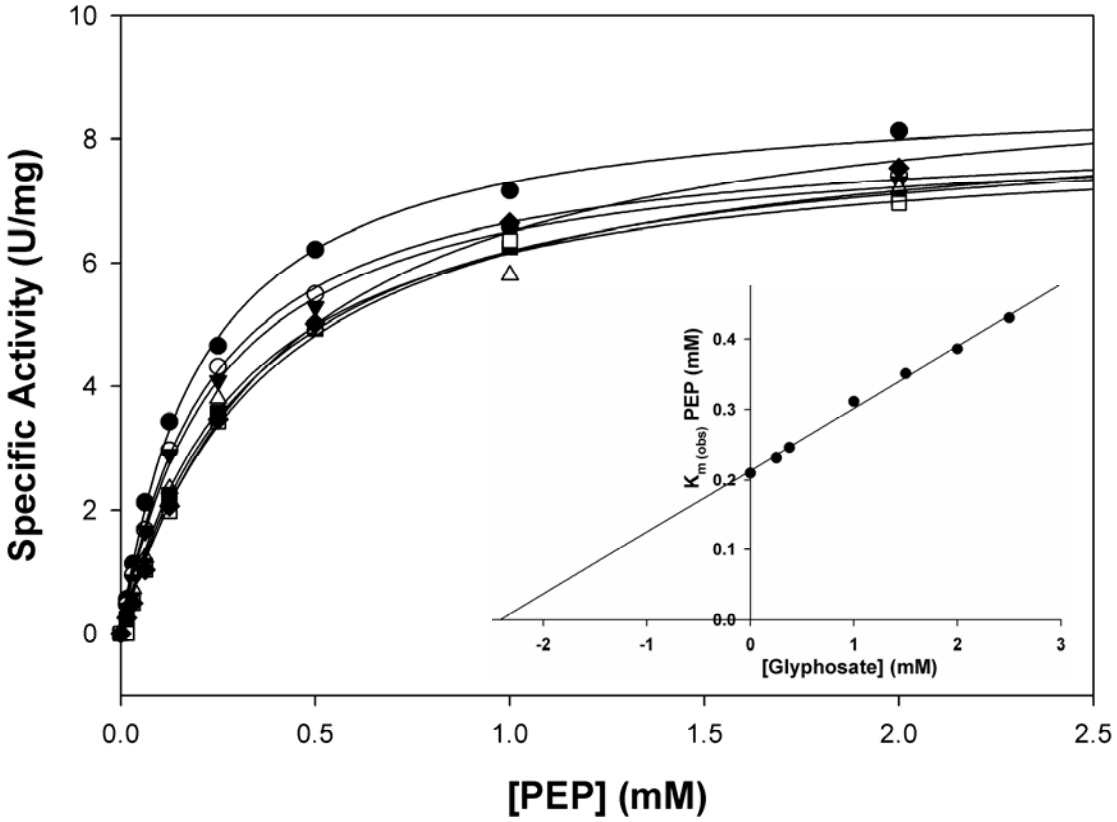


FIGURE S4. **Inhibition of TIPS EPSPS by glyphosate: determination of K_i.**
Substrate saturation in the presence of increasing concentrations of glyphosate: 0 μM (●), 0.25 mM (○), 0.375 mM (▼), 1.0 mM (△), 1.5 mM (■), 2.0 mM (□), and 2.5 mM (◆). S3P concentration was 1 mM. Enzyme concentration was 7.9 nM. Data were fit to the Michaelis-Menten equation.
Inset: Replot of the observed K_m values as a function of glyphosate concentration. Data were fit to equation (1) yielding K_i of 2420 ± 87 μM.

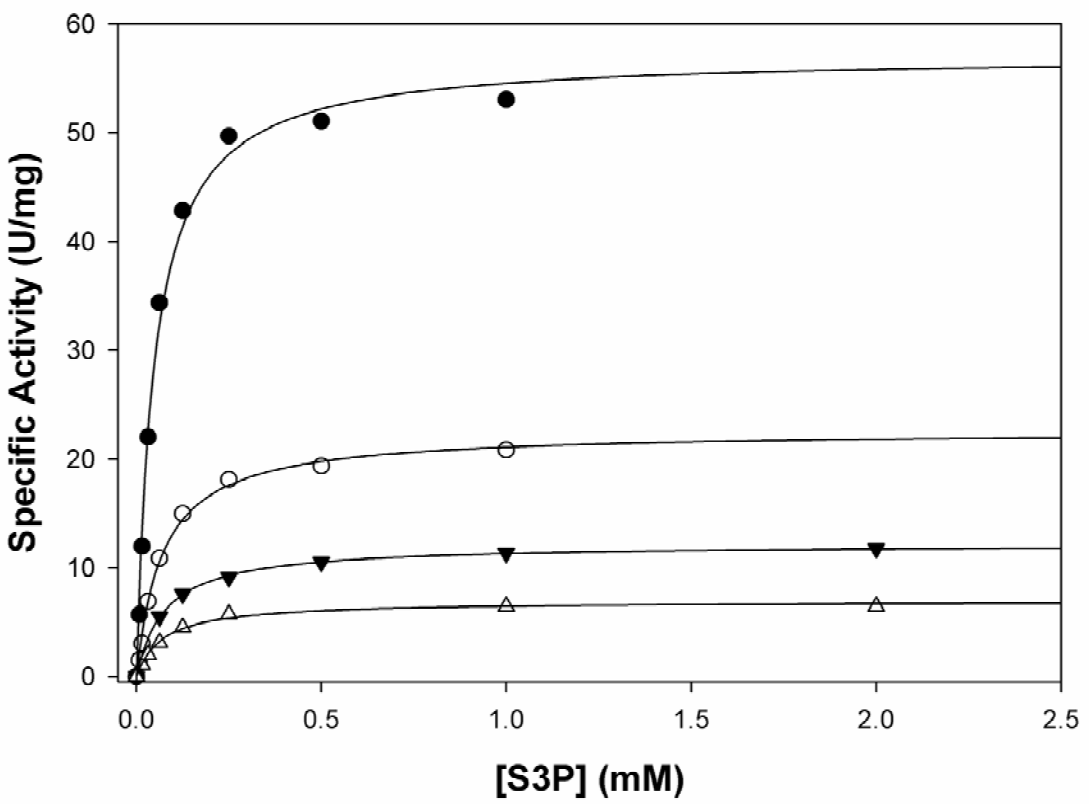


FIGURE S5. **Activity of wild-type and mutant enzymes: determination of the K_m for S3P.** Substrate saturation curves for WT (●), P101S (○), T97I (▼), and TIPS (△) using 1 mM PEP and increasing concentrations of S3P. Data were fit to the Michaelis-Menten equation yielding the K_m values listed in Table 2.

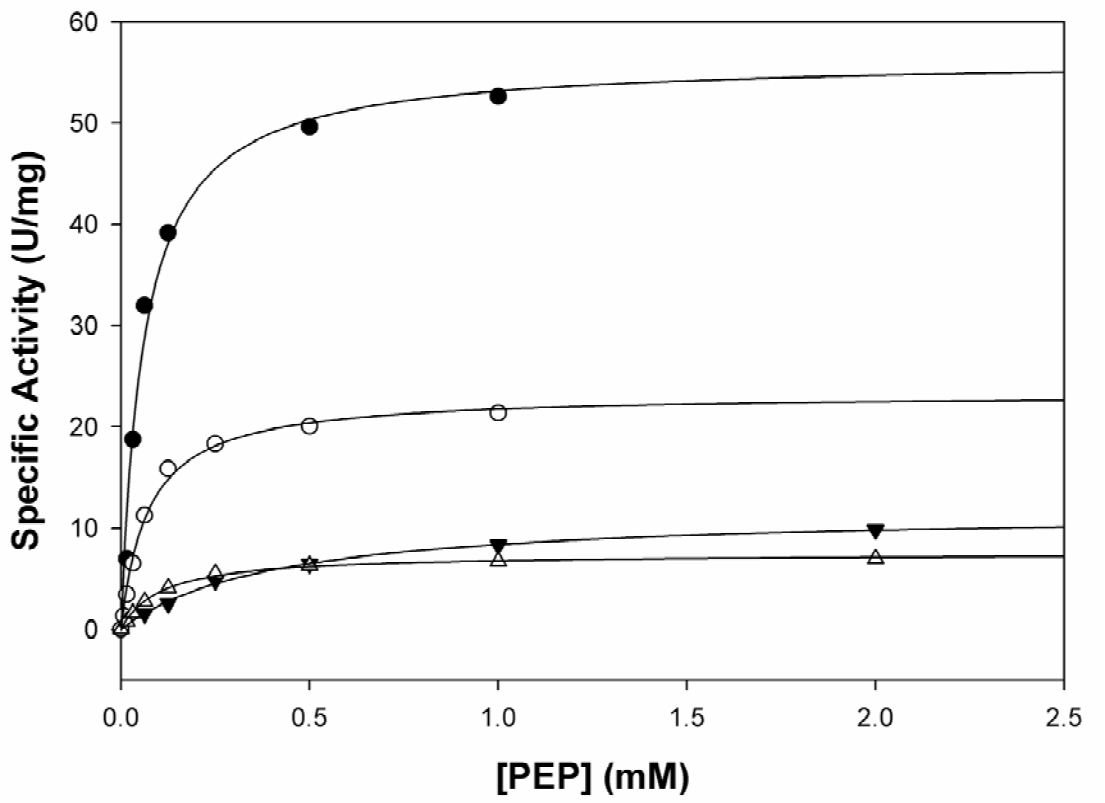
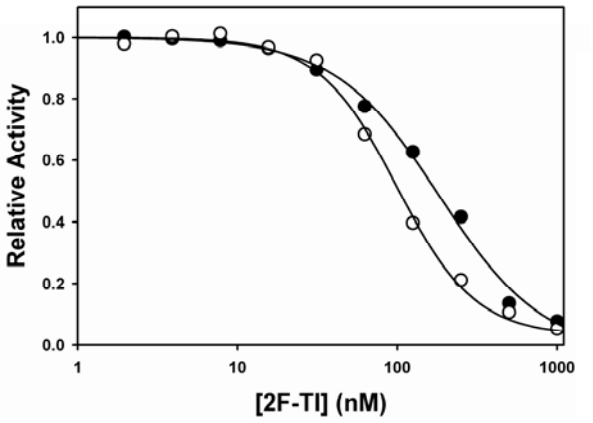
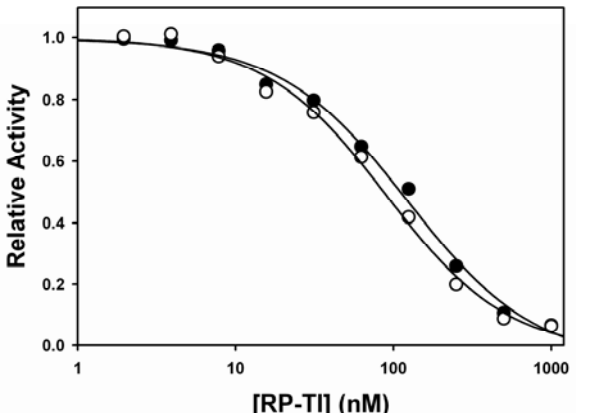


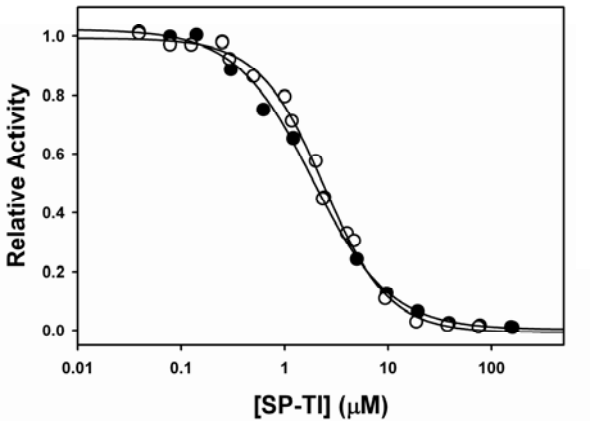
FIGURE S6. **Activity of wild-type and mutant enzymes: determination of the K_m for PEP.** Substrate saturation curves for WT (●), P101S (○), T97I (▼), and TIPS (△) using 1 mM S3P and increasing concentrations of PEP. Data were fit to the Michaelis-Menten equation yielding the K_m values listed in Table 2.



(*Top*) 2F-TI inhibition
IC_{50} TIPS (●) = 180 nM
IC_{50} WT EPSPS (○) = 99 nM



(*Center*) RP-TI inhibition
IC_{50} TIPS (●) = 120 nM
IC_{50} WT EPSPS (○) = 91 nM



(*Bottom*) SP-TI inhibition
IC_{50} TIPS = (●) 1.9 μM
IC_{50} WT EPSPS (○) = 2.4 μM

FIGURE S7. **Inhibition of TIPS and WT EPSPS by analogs of the tetrahedral reaction intermediate (TI).**
Enzymatic activity was determined in the presence of increasing concentrations of the (*R*)-difluoro-TI analog (2F-TI), the (*R*)-phosphonate-TI analog (RP-TI), and the (*S*)-phosphonate-TI analog (SP-TI).

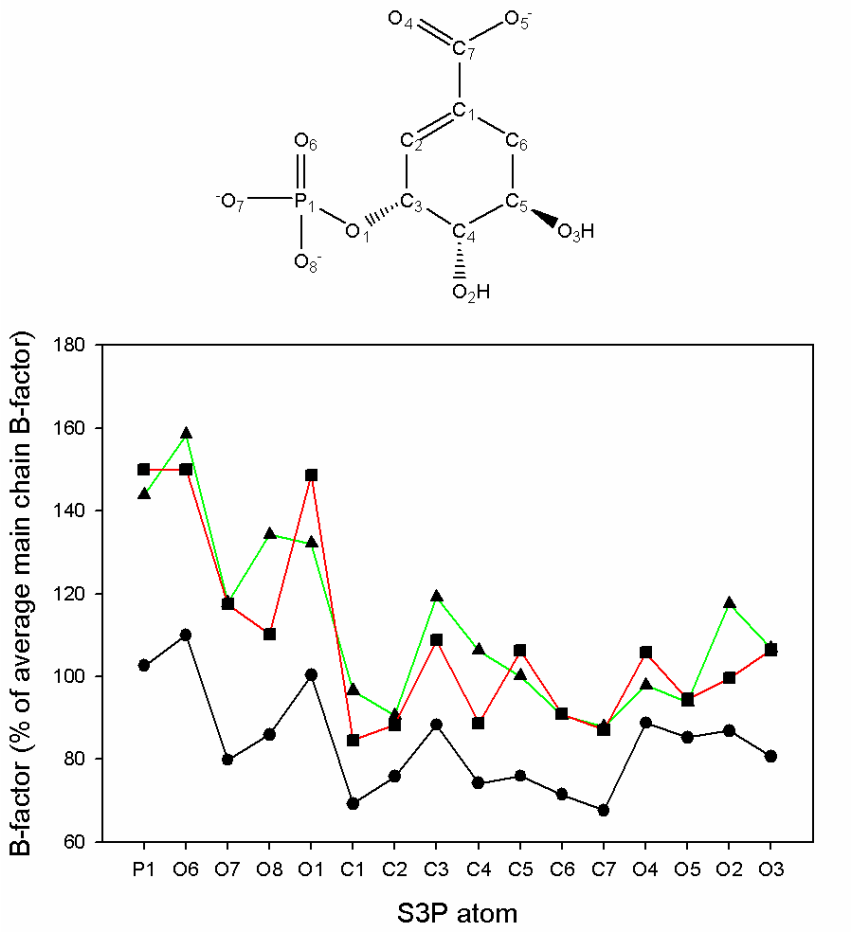


FIGURE S8. **Impact of the mutations on the S3P molecule.**
The average B-factor of the S3P molecule when bound to the T97I (▲) and TIPS (■) enzymes is slightly increased over S3P bound in the WT enzyme (●).