

SUPPLEMENTARY MATERIAL

Table 1S: Kinetic parameters of the pools of WT and mutant enzyme obtained by gel filtration chromatography at 25 °C ^a

Enzyme	Column Fractions (Figure 5)	Double reciprocal plot ^b				Hill equation ^c		
		K_m^{-1} (μM)	V_m^{-1} ($\mu\text{mol}/\text{min}/\text{mg}$)	K_m^{-2} (μM)	V_m^{-2} ($\mu\text{mol}/\text{min}/\text{mg}$)	$V_{\text{max}} \pm \text{S. E}$ ($\mu\text{mol}/\text{min}/\text{mg}$)	$K_{0.5} \pm \text{S. E}$ (μM)	Hill Coefficient ($n \pm \text{S. E}$)
WT	35-38					7.5 ± 0.3	1.9 ± 0.05	2.0 ± 0.2
	Overall					8.5 ± 0.4	2.1 ± 0.3	1.9 ± 0.1
K246E	26-31	0.2	0.17	0.9	0.21	0.23 ± 0.02	1.1 ± 0.2	0.5 ± 0.05
	32-36			0.8	0.25	0.25 ± 0.01	0.9 ± 0.1	0.9 ± 0.2
	overall	0.2	0.18	0.8	0.23	0.23 ± 0.03	0.8 ± 0.2	0.5 ± 0.1

^a The V_{max} and K_m values were determined by measuring the initial velocity as the concentration of SAMP was varied. ^b The data were plotted using double reciprocal plots. ^c The V_{max} , $K_{0.5}$ and Hill coefficients were obtained by fitting the data to the Hill equation using Sigma Plot. The values are shown along with their standard errors.

FIGURE LEGENDS OF SUPPORTING FIGURES

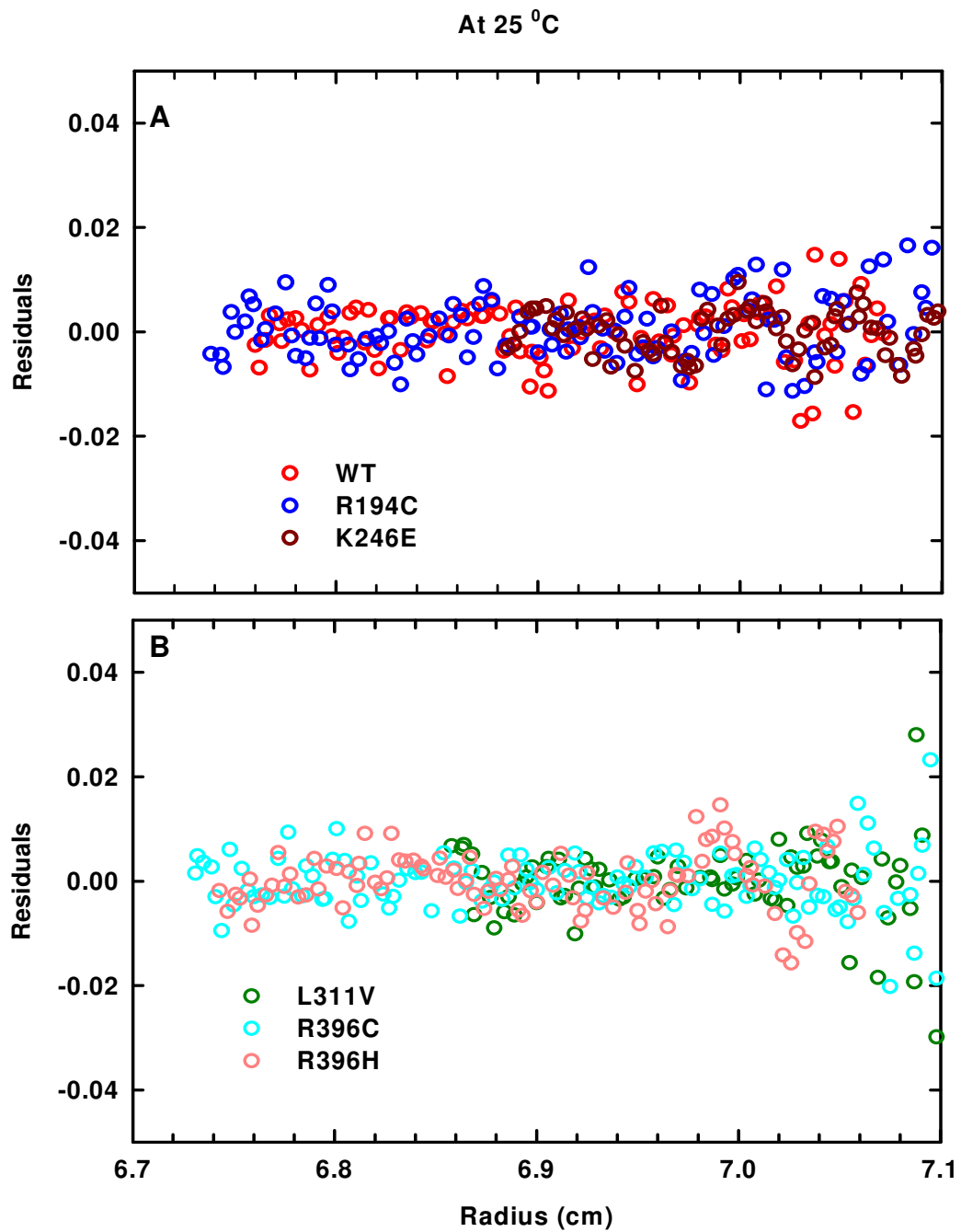
FIGURE 1S: Representative experimental AUC residual data of the WT and mutant enzymes at 25 °C. None of the enzymes has a good fit to three state theoretical models; they only exhibit good fits to two state theoretical models. M = monomer, D = dimer, Tet = tetramer.

(A) Residuals of the WT, R194C, and K246E enzymes (~0.34 mg/ mL) at 25 °C; the best fit model for WT and R194C is the M–Tet theoretical model, whereas the K246E mutant enzyme exhibits good fits with D–12mer and M–9mer theoretical models, but only the residuals of D–12mer theoretical model are represented. (B) Residuals of the L311V, R396C, and R396H enzymes (~0.34 mg/ mL) at 25 °C where L311V and R396C exhibit good fits only with the M–Tet theoretical model and R396H has a good fit with the D–Tet theoretical model.

FIGURE 2S: Representative experimental AUC residual data of the WT and mutant enzymes at 37 °C. None of the enzymes has a good fit to three state theoretical models; they only have good fits with the two state theoretical models. M = monomer, D = dimer, Tet = tetramer.

(A) Residuals of the WT, R194C, and K246E enzymes (~0.34 mg/ mL) at 37 °C; the best fit model for WT and R194C is the M–Tet theoretical model, whereas the K246E mutant enzyme has a good fit with the M–14mer theoretical model. (B) Residuals of the L311V, R396C, and R396H enzymes (~0.34 mg/ mL) at 37 °C, where all exhibit good fits with the M–Tet theoretical model, while R396C and R396H also fits with the D–5mer theoretical model.

Supporting Figure 1S



Supporting Figure 2S

