SUPPLEMENTAL INFORMATION

The Time-course of Aldehyde Oxidase and Why it is Nonlinear

Armina Abbasi, Erickson M. Paragas, Carolyn A. Joswig-Jones,

John T. Rodgers, and Jeffrey P. Jones

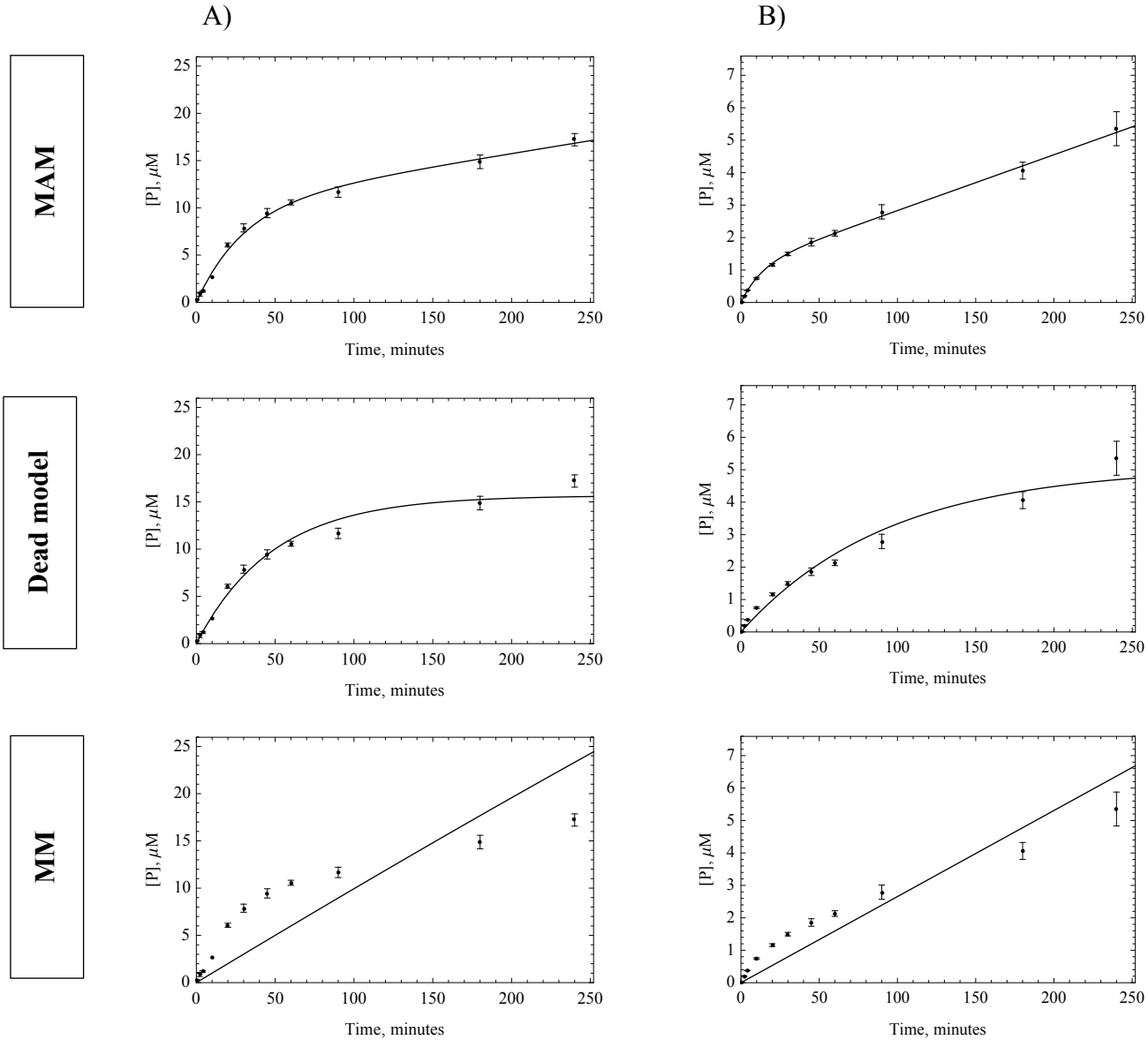
Department of Chemistry, Washington State University, Pullman, WA 99164-4630

Drug Metabolism and Disposition

Supplemental Table 1. Comparison between the rate constants obtained from the fits to the substrate inhibition models and the MAM.

substrate	rate constants	SI 1	SI 2	SI 3	MAM
DACA	k3 (1/min)	23.35	28.63	80.24	22.96
	k4 (1/min)	0.04	0.05	---	0.019
	k5 (1/min)	2.12	2.15	---	3.46
phthalazine	k3 (1/min)	167.2	200.99	265.71	168.63
	k4 (1/min)	0.97	1.16	---	0.49
	k5 (1/min)	3.15	3.15	---	5.25

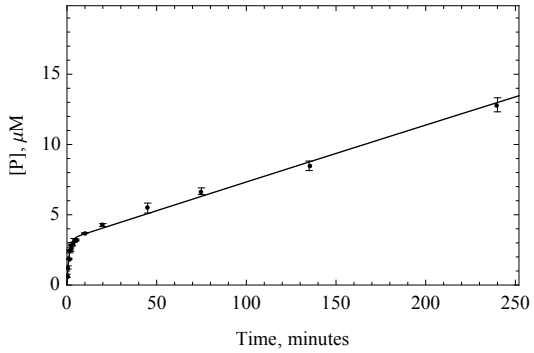
Supplemental Fig. 1. The time-course data for different substrates of aldehyde oxidase, A) DACA, B) zaleplon, C)phthalazine, D) BIBX1382, E) zoniporide, were fit to three different kinetic models namely, modulated activity model (MAM), dead model, and Michaelis-Menten. The goodness of fit was judged to be the best to the MAM (with the exception of zoniporide) by the Akaike value (AIC).

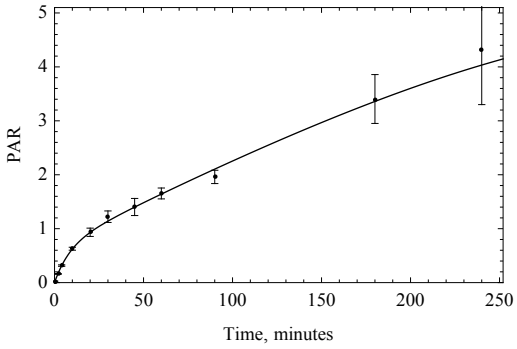


C)

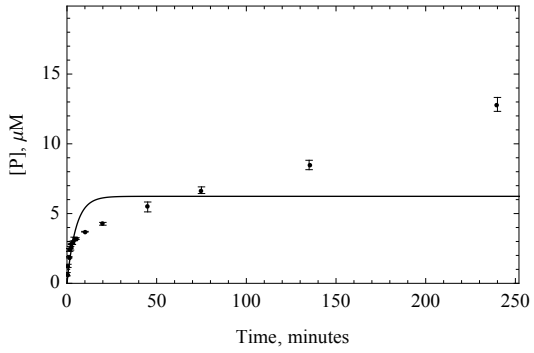
D)

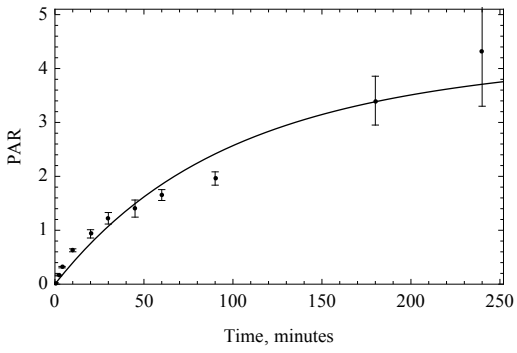
MAM



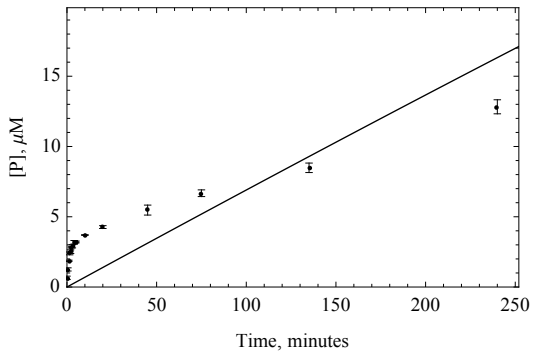


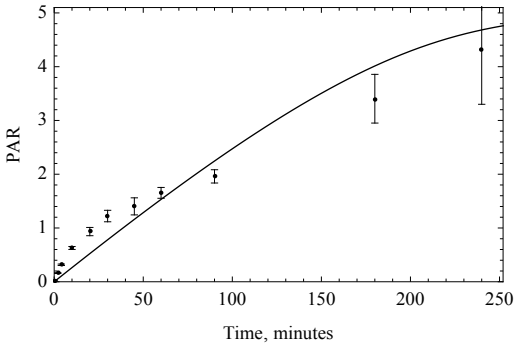
Dead model



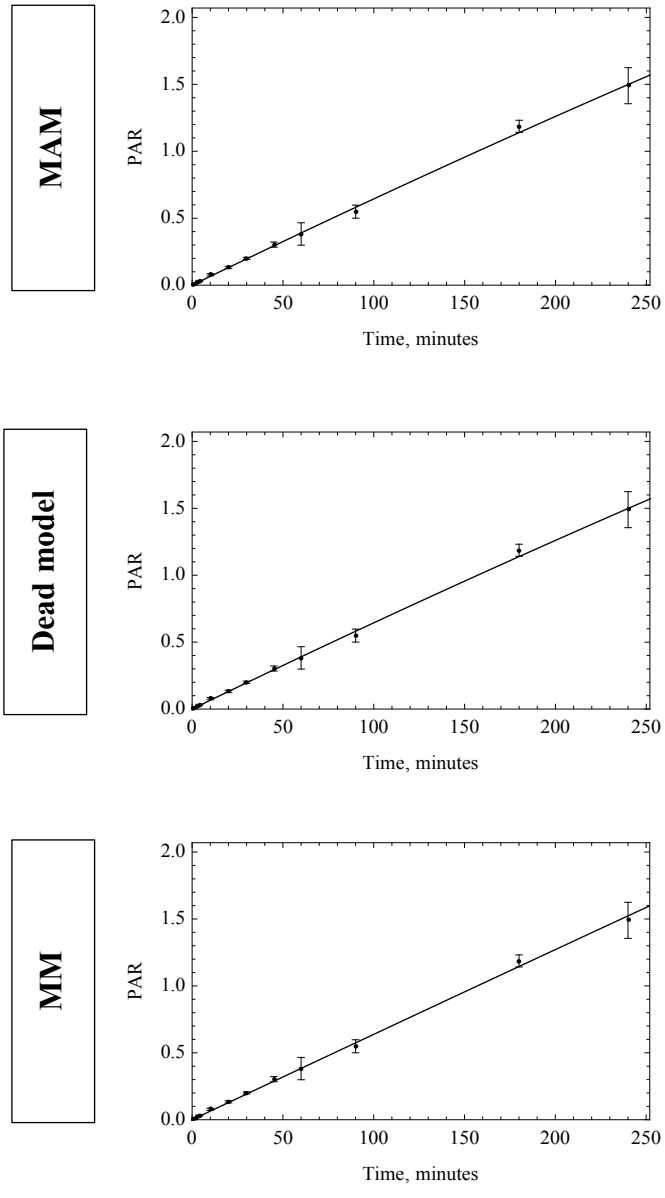


MM



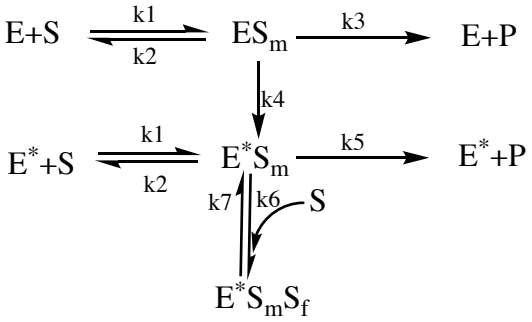


E)

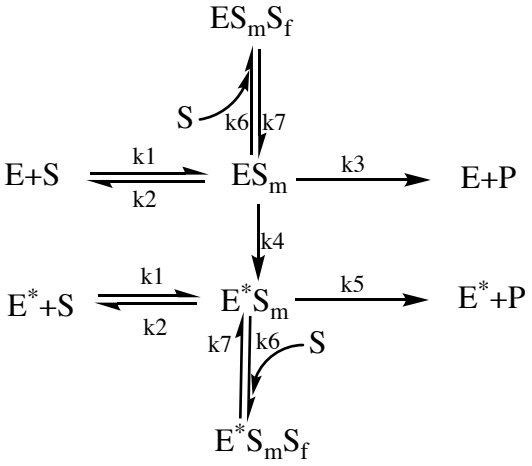

MAM
PAR
Time, minutes
Dead model
PAR
Time, minutes
MM
PAR
Time, minutes

Supplemental Fig. 2. Kinetic scheme of substrate inhibition. The subscripts m and f refer to the Moco site and the flavin site respectively since we are assuming that the second substrate goes to the flavin site. We have set $k_7 = k_1$ with the exception of model 3 in which k_6 was set to a value closer to the initial guess for k_3 to get a better fit. In all the models k_6 was set equal to $K_I \times k_7$.

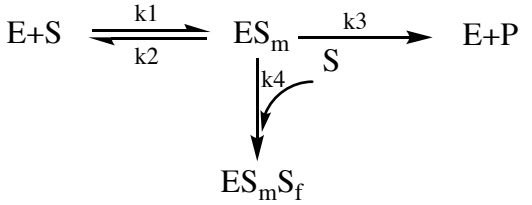
A) Substrate inhibition model 1 (SI 1)



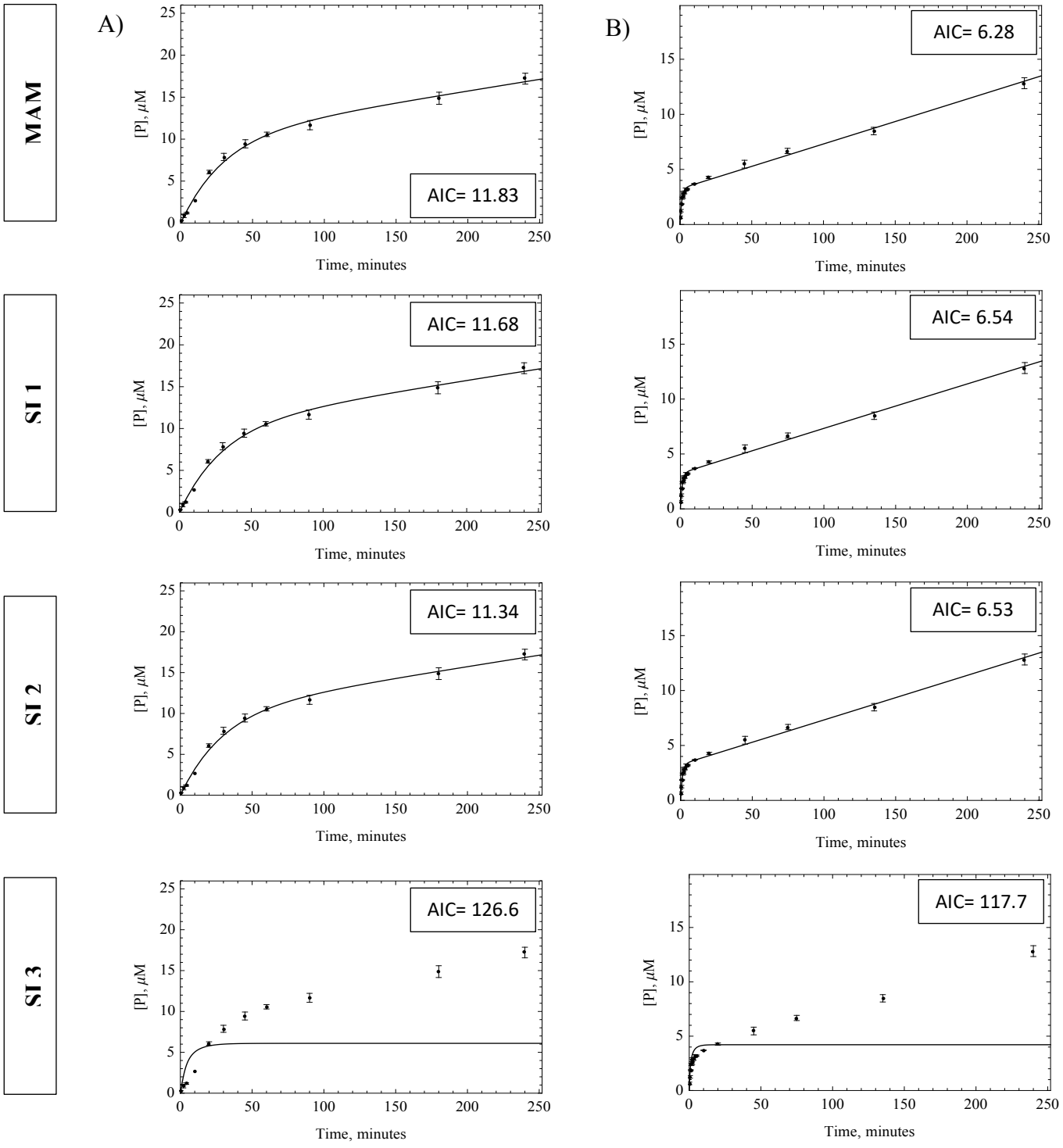
B) Substrate inhibition model 2 (SI 2)



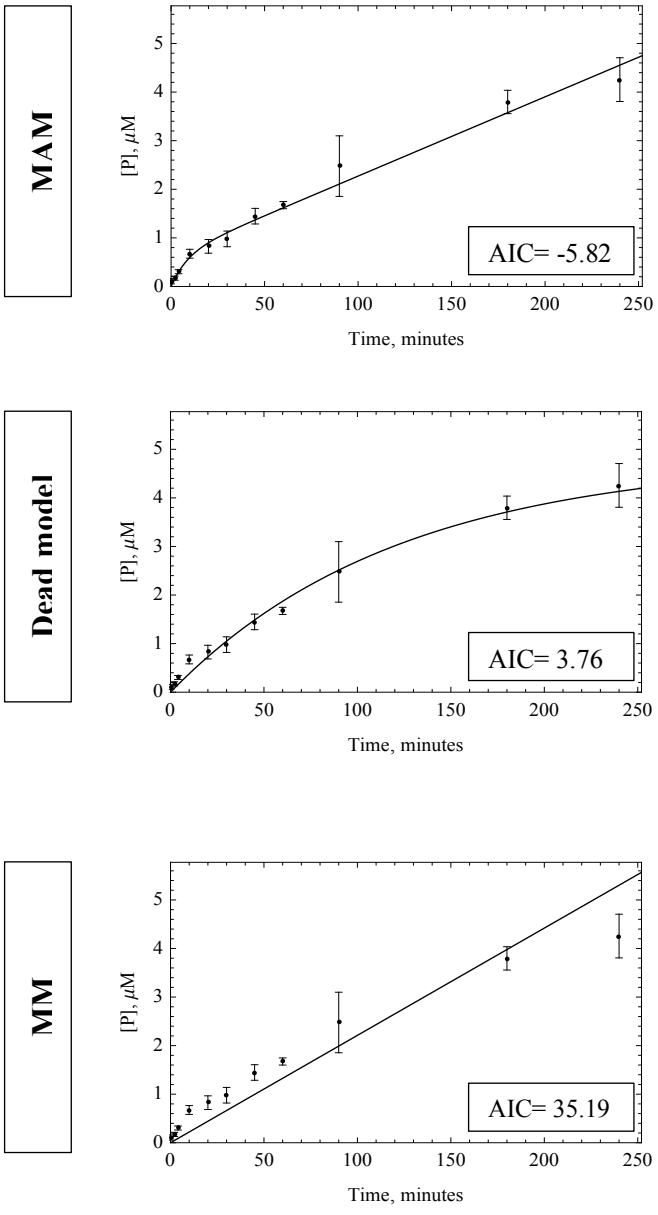
C) Substrate inhibition model3 (SI 3)



Supplemental Fig. 3. Comparing the fits from the MAM and the substrate inhibition models to the two substrates A) DACA and B) phthalazine that exhibit substrate inhibition. Based on the AIC values, the substrate inhibition model does not necessarily provide a better fit.



Supplemental Fig. 4. The time course plot of O6BG in human liver S9 fraction shows that the nonlinear behavior of the enzyme persists even in a more intact cell machinery. The data points were fit to the three kinetic models, MAM, Dead model, and MM, (n=3, P< 0.001).



Supplemental Fig. 5. In vivo intrinsic clearance vs. in vitro intrinsic clearance calculated based on the MAM model shows a linear correlation for the three substrate (O6BG, DACA, zaleplon) used for the in vitro calculations. This linearity may refer to the probability of involvement of extrahepatic clearance by AOX.