

Supplemental Information

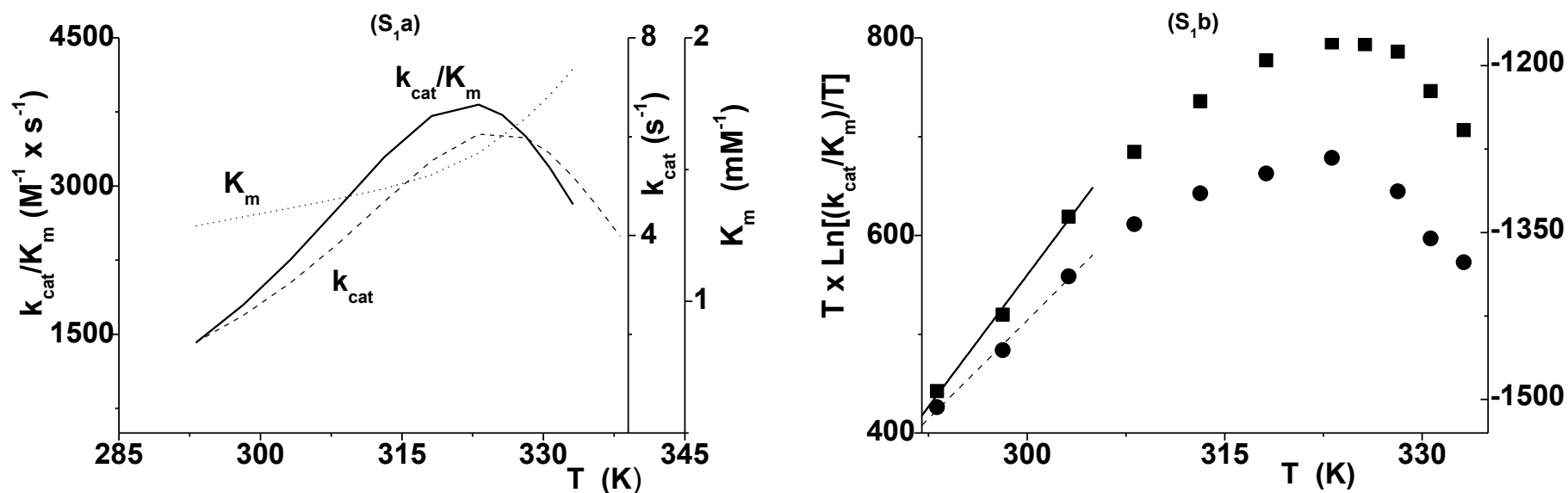


Figure S1: (a) Dependencies of Michaelis-Menten parameters k_{cat}/K_m , k_{cat} , and $1/K_m$, vs. the absolute temperature; (b) Eyring plots of parameters k_{cat}/K_m and k_{cat} . The values of rate constants, their activation energies (A^E) and the thermodynamic parameters of activation were estimated i.e. $k_1=5457\pm 40 M^{-1} s^{-1}$, $A^E_{k_1}=35.4\pm 0.3 kJ mol^{-1}$, $k_2=8150\pm 61 s^{-1}$, $A^E_{k_2}=141.5\pm 0.2 kJ mol^{-1}$, $k_3=19930\pm 144 s^{-1}$, $A^E_{k_3}=8.9\pm 0.2 kJ mol^{-1}$, $k_4=5589\pm 29 s^{-1}$, $A^E_{k_4}=144.5\pm 0.2 kJ mol^{-1}$, $\Delta H^\ddagger_{k_{cat}/K_m}=39.70 kJ mol^{-1}$, $\Delta S^\ddagger_{k_{cat}/K_m}=-49.69 J mol^{-1} K^{-1}$, $\Delta G^\ddagger_{k_{cat}/K_m}=55.76 kJ mol^{-1} K^{-1}$, $\Delta H^\ddagger_{k_{cat}}=41.28 kJ mol^{-1}$, $\Delta S^\ddagger_{k_{cat}}=-99.53 J mol^{-1} K^{-1}$, $\Delta G^\ddagger_{k_{cat}}=73.45 kJ mol^{-1}$.

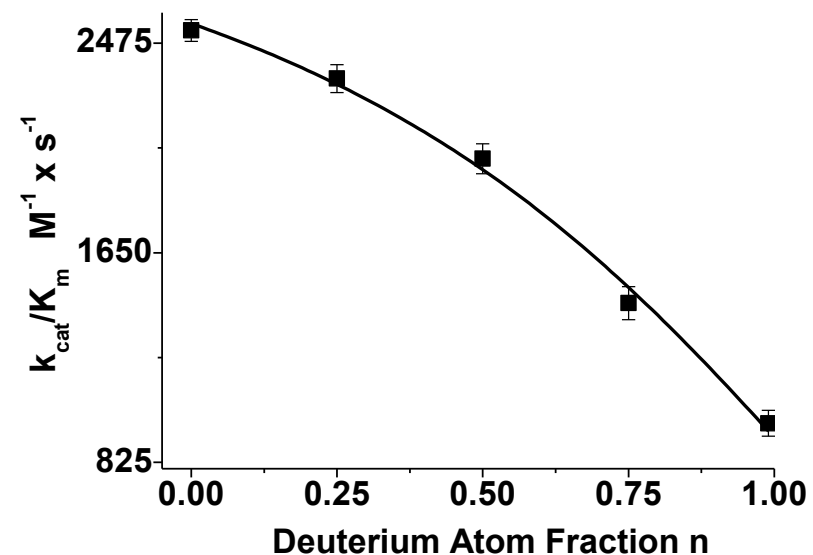


Figure S2: Bowed-upward proton inventories of k_{cat}/K_m ; the experimental data were best fitted by the equation

$$k^n = k^0 \frac{Z_1^n}{C_{\text{Ph}} + \frac{C_{\text{Ch}}}{(1-n+n\phi^{\text{T,Ch}})^\mu}},$$

where k^n , k^0 , correspond to parameter k_{cat}/K_m in D_2O atom fraction n and in H_2O , respectively, Z_1

is a transition-state fractionation factor state showing reorganization of solvent during the substrate binding, C_{Ph} and C_{Ch} are transition-state fractionation factors of physical and chemical steps, respectively, $\phi^{\text{T,Ch}}$ is a transition-state fractionation factor expressing the exchangeable sites of charge-relay system, μ denotes the number of the transferred protons.

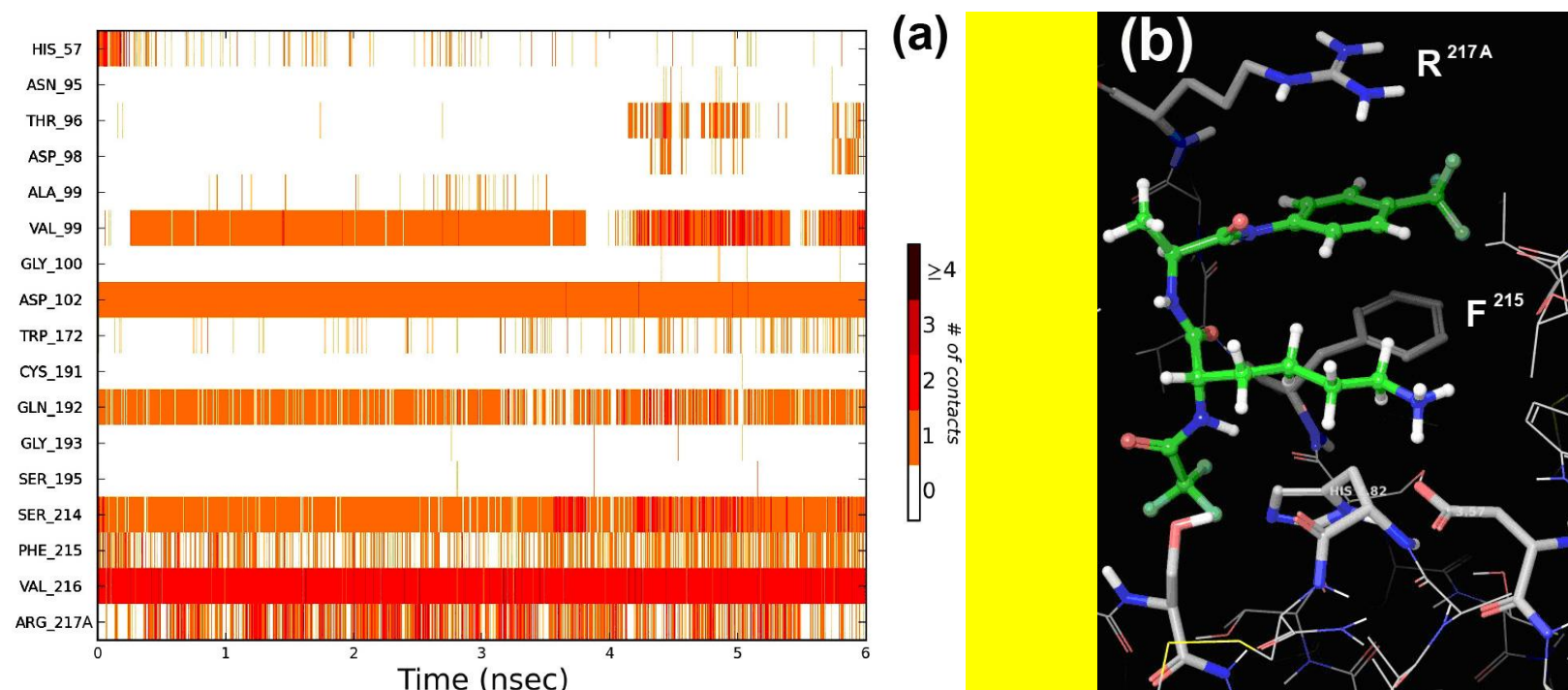


Figure S3: Interactions of PPE and inhibitor-1 during the MD simulation: (a) interactions of PPE-backbone residues and bound inhibitor (PDB ID: 7EST) vs. 5 ns time, (b) snapshot from the MD simulation (the C-terminus -C₆H₄-CF₃ group of inhibitor-1 is found among the residues F²¹⁵ and R^{217A} of PPE-backbone).

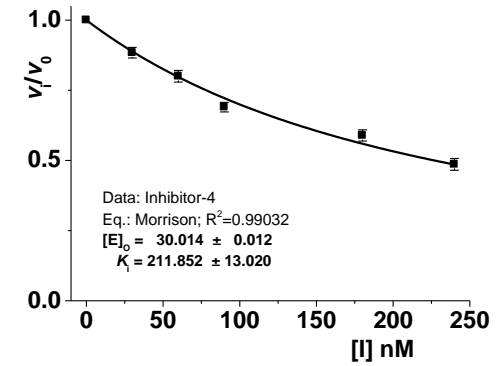
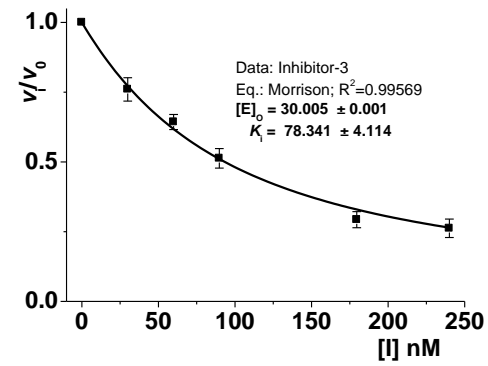
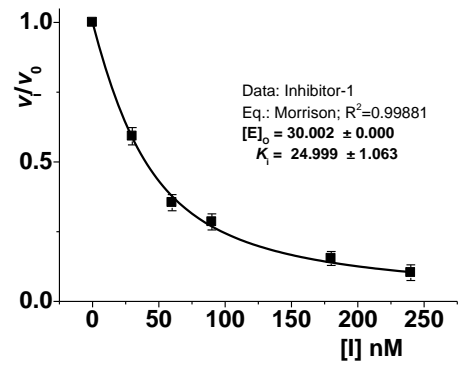


Figure S4: Determination of the parameter K_i for inhibitors-1,3,4. The experimental conditions are described in the text.

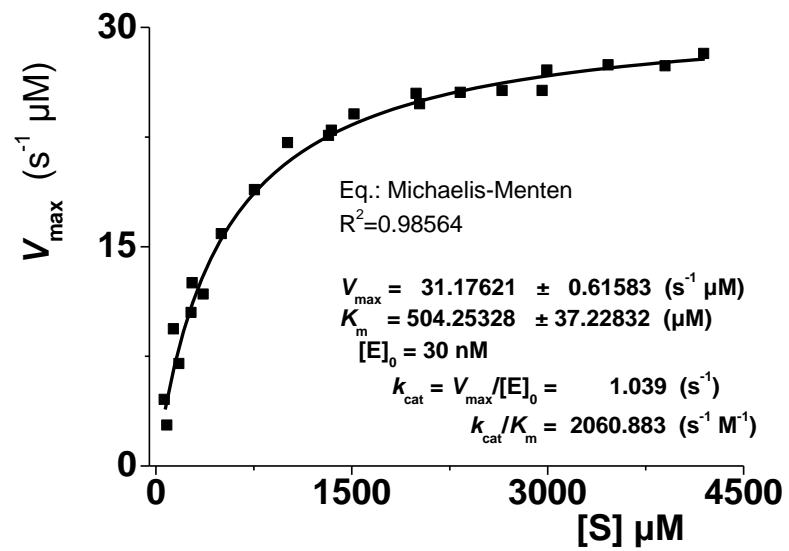


Figure S5: Michaelis-Menten curve of the hydrolysis of substrate Suc-AAA-pNA by PPE, in buffer 0.1 M Tris-HCl, pH 8.0, at 25°C.

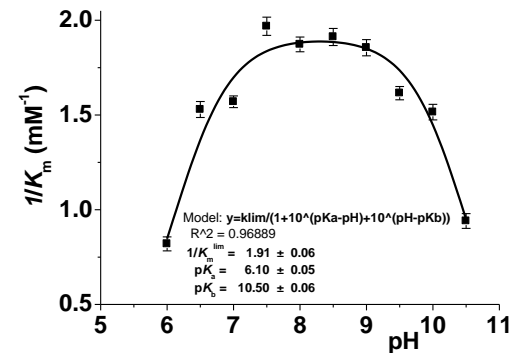
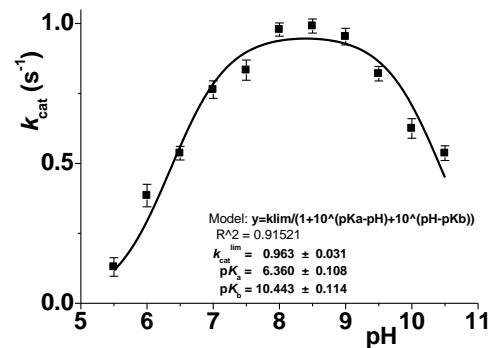
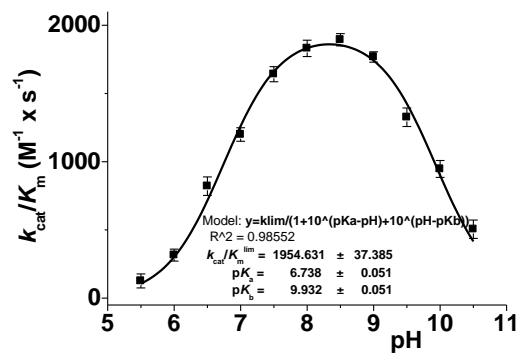


Figure S6: Dependencies of the parameters k_{cat}/K_m , k_{cat} , and $1/K_m$ versus pH, in different buffers of 0.1M ionic strength, at 25°C.

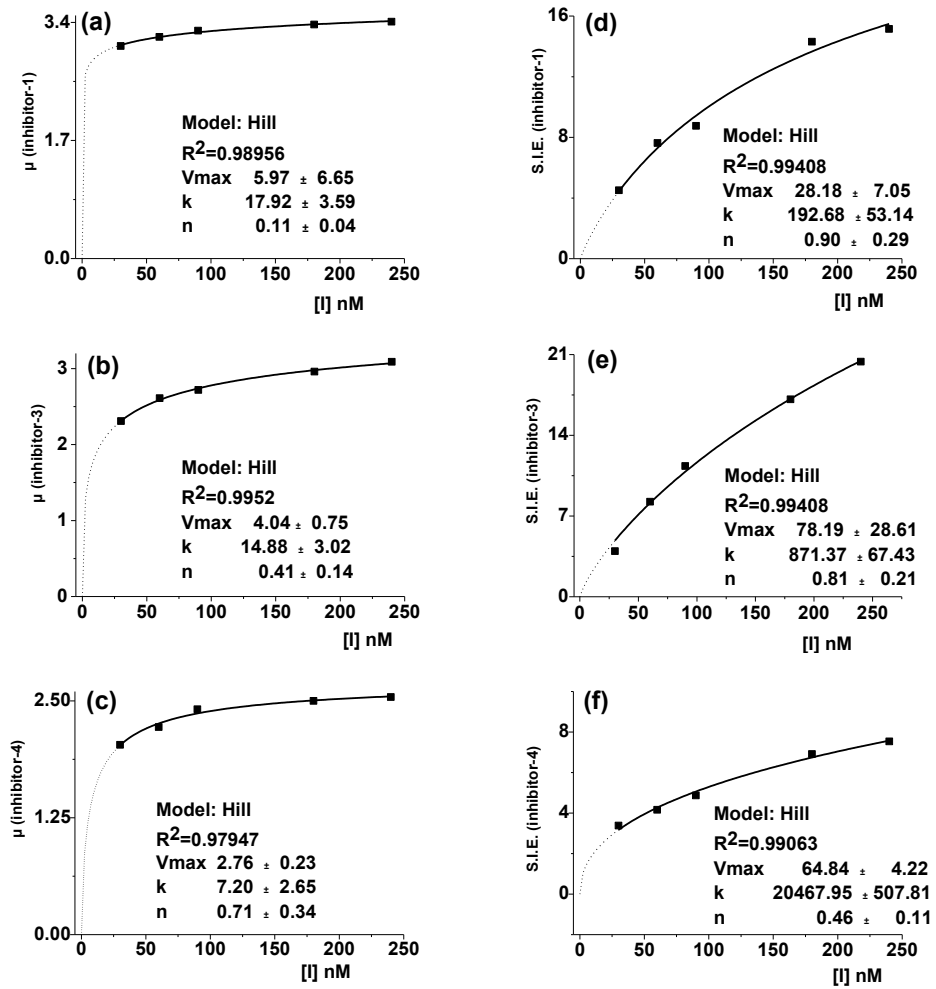


Figure S7: Fitting of data from TABLE 3 (inhibitor-1, inhibitor-3, and inhibitor-4), concerning the exponents μ (curves a,b,c) and the S.I.E. (curves d,e,f) by the Hill equation; the Hill coefficient n is a measure of the negative cooperativity.

