Supporting information for: Allosteric-Activation Mechanism Of Bovine Chymosin Revealed By Bias-Exchange Metadynamics and Molecular Dynamics Simulations

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Tyr77 dihedral analysis

The χ_{77} dihedral angle in Tyr77 as a function of simulation time is shown in Figure S1 (for the Amber ff03 simulation of the C3 complex). See the main article for further discussion.

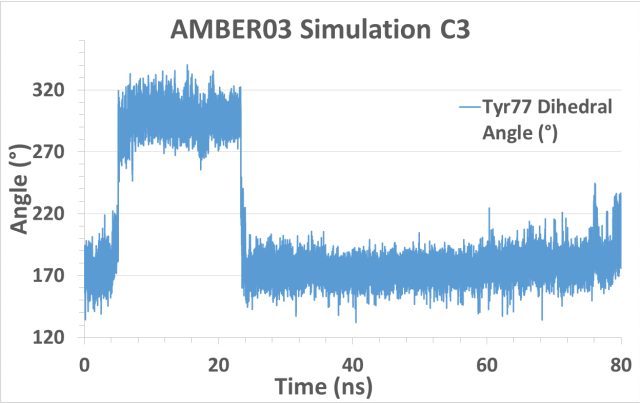

Figure S1: Tyr77 dihedral angle (blue) - AMBER03 Selfinhibited-HPHPH protonated complex (C3).

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Tyr77 and Phe114 interactions

Apo-Chymosin

In the simulations of apo-chymosin, Tyr77 makes close contacts with two residues in the α-helix, Val113 and Phe114. Phe114 extends towards the β-hairpin flap of chymosin when Tyr77 is in its self-inhibited state and extends away from the flap when Tyr77 is in its open conformation. In the *A1* simulation, in which Tyr77 is in the open conformation, contact with Phe114 occurs for 55% of the trajectory (using a distance of 4 Å between any non-hydrogen atoms to define a contact in the AMBER ff99SB-ILDN simulation). This increases to 94% in simulation *B1* where Tyr77 is in the self-inhibited conformation.

Chymosin – P8-P4 κ-Casein

In simulation *C1* using AMBER ff99SB-ILDN, Tyr77 remains in contact with the Phe114 in both its selfinhibited (73%) and open conformations (78%). There is an increase in atom-atom contacts between Tyr77.N:Phe114.CZ (15% $\rightarrow$ 36%) and Tyr77.H:Phe114.CE2 (6% $\rightarrow$ 11%) after the self-inhibited to open conformation. Phe114 makes

fewer contacts with Trp41 (which is located under the β-hairpin flap in the binding pocket) when Tyr77 is in its open conformation (37% $\rightarrow$ 17%).

In simulation *C2* using the AMBER03 force field, Tyr77 remains in contact with residue Phe114 in both the self-inhibited (67%) and open (74%) conformations. Close contacts are shown to increase for the same atoms as in the previous simulations when Tyr77 moves to its open state, Tyr77.N:Phe114.CZ (11% $\rightarrow$ 39%) and Tyr77.H:Phe114.CE2 (5% $\rightarrow$ 24%). Contact between Trp41 and Phe114 is also seen to decrease when Tyr77 is in its open state (66% $\rightarrow$ 32%).

Simulation *C3* using the AMBER03 force field contains a short 17ns period where Tyr77 changes to its open form before returning to the self-inhibited state. Residue contacts analysis reveals Tyr77 and Phe114 remains in contact in both the selfinhibited (84%) and open (80%) conformations. Atom contacts follow the same trend as seen in the simulations described above with an increase when Tyr77 is in its open conformation for Tyr77.N:Phe114.CZ (11% $\rightarrow$ 39%) and Tyr77.H:Phe114.CE2 (5% $\rightarrow$ 24%). However analysis of the residue contact between Trp41 and Phe114 reveal that there is an increase when Tyr77 is in its open conformation (selfinhibited-35% $\rightarrow$ open-51%).

Hydrogen-Bonding

Table S1: Percentage of total simulation time in which specific hydrogen bonds were observed in the Bias-Exchange Metadynamics simulations

		Apo Enzyme			Holo Enzyme		
Donor	Acceptor	Closed	Intermediate	Open	Closed	Intermediate	Open
ASN10	GLY161	63	57	94	1	2	2
ASN10	ASP158	9	17	96	66	26	53
SER164	ASN10	74	59	3	93	61	93
SER220	ASP13	48	54	93	0	0	0
ARG304	ASP13	37	33	95	68	18	50
GLU118	GLN15	97	97	73	0	4	13
GLN15	GLY218	0	0	0	74	63	70
TYR16	TYR156	64	82	92	0	0	0