

Supplemental Information

Explicit Treatment of Non Michaelis-Menten and Atypical Kinetics in Early Drug Discovery

Bharath Srinivasan^{#,1}

[#] Mechanistic Biology and Profiling, Discovery Sciences, R&D, AstraZeneca, Cambridge, UK

¹bharath.srinivasan@astrazeneca.com; +44 7508382559

Figure S1. Graphical method to understand Mechanism of inhibition for enzymes showing substrate cooperativity. Competitive inhibition and positive cooperativity are highlighted as specific case studies but the approach is applicable to all mechanisms of inhibition and for negative cooperative systems too. **(A)** Competitive inhibition of a system with no cooperativity for substrate. **(B)** double-reciprocal LB plots showing intersection on the Y-axis diagnostic of competitive inhibition. **(C)** Competitive inhibition of a system with positive cooperativity for substrate. **(D)** non-linearity of the double-reciprocal LB plots make it complex to assign inhibition modality. **(E)** transformation of the substrate by raising it to the power of Hill coefficient to get back the linear LB plots facilitating unambiguous assignment of inhibition MoA. The plots were generated from the respective equations of competitive inhibition with and without inbuilt cooperativity. The following parameters were fixed: V_{max} -10, K_m -5, K_i =5, n =2, I = 0, 5, 10, 20 and 40, respectively. The units were arbitrarily fixed as μM for concentration and minutes for time.

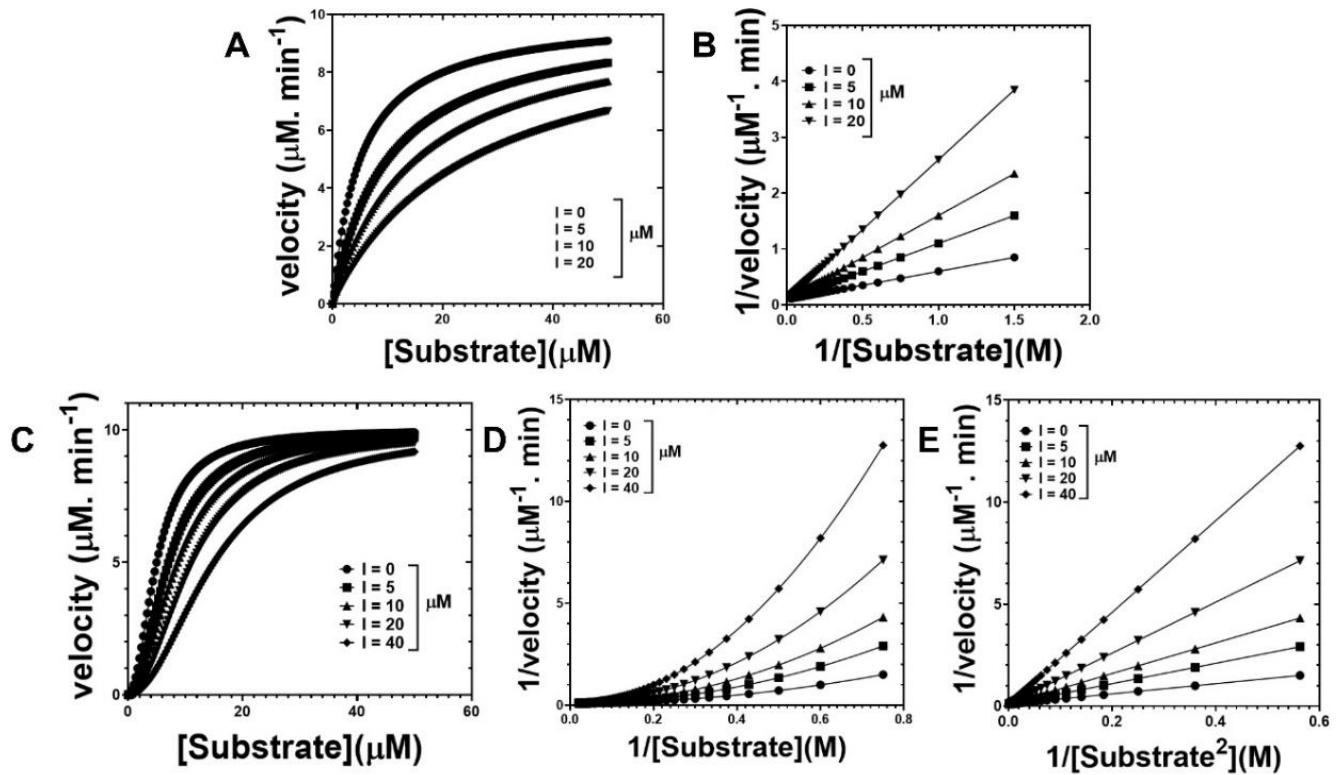


Figure S2. Diagnostic Ackermann-Potter plots for understanding (A) reversible inhibition (B) irreversible inhibition and (C) tight-binding inhibition.

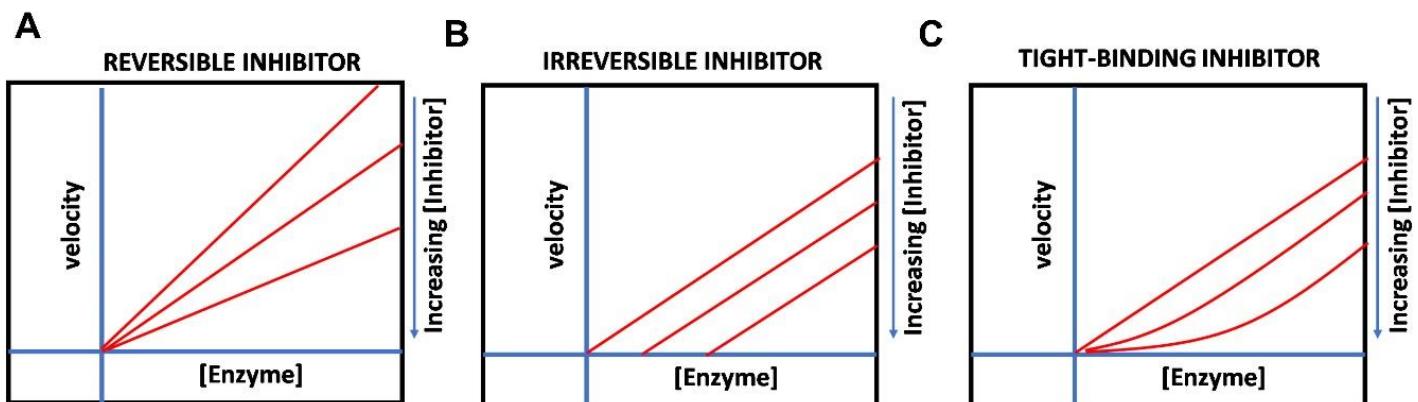


Figure S3. Covalent irreversible inhibition **(A)** Primary plot of covalent inhibition versus product formation where the inhibition sets in as a function of time before complete inhibition is achieved. **(B)** secondary replot of k_{obs} versus inhibitor concentration for single step inhibition and **(C)** secondary replot of k_{obs} versus inhibitor concentration for two step inhibition¹³⁸.

