

The role of substrate unbinding in Michaelis–Menten enzymatic reactions

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Short Abstract — The Michaelis–Menten equation provides a hundred-year-old prediction by which any increase in the rate of substrate unbinding will decrease the rate of enzymatic turnover. Surprisingly, this prediction was never tested experimentally nor was it scrutinized using modern theoretical tools. We explain why, and under what circumstances, an increase in the rate at which an enzyme unproductively departs from a bound substrate will—unexpectedly—lead to accelerated product formation. Our findings are independent of the enzymological context within which they are presented and relevant to any system described by a stochastic Michaelis–Menten reaction scheme.

I. INTRODUCTION

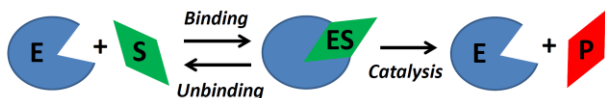


Figure 1: An illustration of the Michaelis-Menten reaction scheme

EVERY enzymatic reaction is composed out of two basic steps: (i) the reversible binding of a substrate molecule to the enzyme, and (ii) a catalytic step which gives rise to the formation of the product. Controlled variation of the effective binding rate to a free enzyme is attained by altering the concentration of the substrate. The rate of product formation (turnover rate) can then be measured as a function of this concentration to show a characteristic hyperbolic dependence. The Michaelis–Menten equation (MME) is used in order to rationalize this observation and interpret the results [1]. Surprisingly, and despite inherent stochasticity, hyperbolic dependence is also observed at the single molecule level [2]. The theoretical foundations behind this (almost) universal behavior (the reason why it is independent of microscopic details) are now well established [3] and one can thus safely say that the role of binding in Michaelis–Menten enzymatic reactions is well understood.

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II. RESULTS

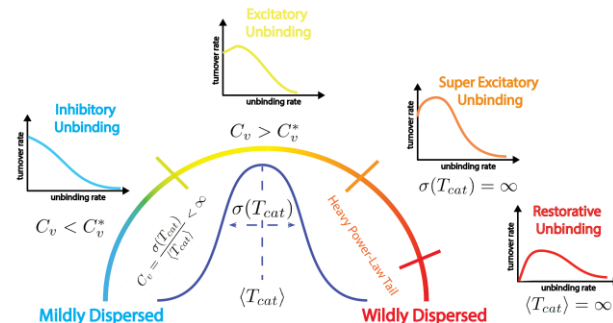


Figure 2: Schematic illustration of the four possible roles of unbinding.

The MME states that any increase in the unbinding rate will decrease the rate of enzymatic turnover. We show that unbinding may also speed up enzymatic turnover — turning a spotlight to the fact that its actual role in enzymatic catalysis remains to be determined experimentally [4]. Analytically constructing the unbinding phase-space, we identify four distinct categories of unbinding: *inhibitory*, *excitatory*, *super-excitatory* and *restorative*. The statistical dispersion in catalysis times (quantified by the coefficient of variation) stands at the heart of our classification and its value (compared to a critical threshold) is shown to determine the role of unbinding. Most importantly, we show that as substrate concentrations increase, a tipping point can be reached where an increase in the unbinding rate results in an increase, rather than a decrease, of the reaction rate. An overlooked tradeoff between the speed and efficiency of enzymatic reactions is naturally unveiled as a result. Our findings indicate that unbinding may behave in ways that violate the classical Michaelis-Menten equation and play a non-trivial role in the facilitation of enzymatic reactions.

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