Supplementary information

On Catalytic Kinetics of Enzymes

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Supplementary Figures

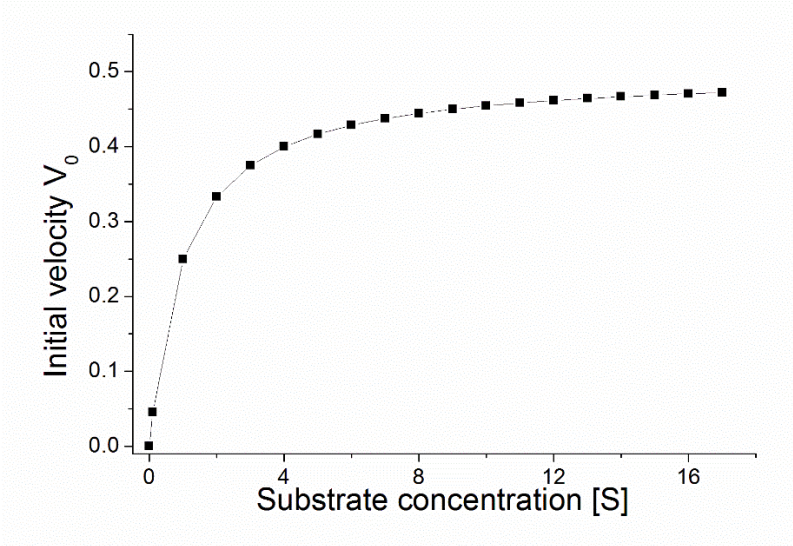

Fig.S1. The shape of Michaelis-Menten equation $V_0=V_{max}[S]/(K_m+[S])$, the relationship between the initial velocity V_0(as Y axis) and the substrate concentration [S] (as X axis) of many enzyme catalyzed reactions show this phenomenon, the function can be changed to the form of $1/y=(a/x)+b$.

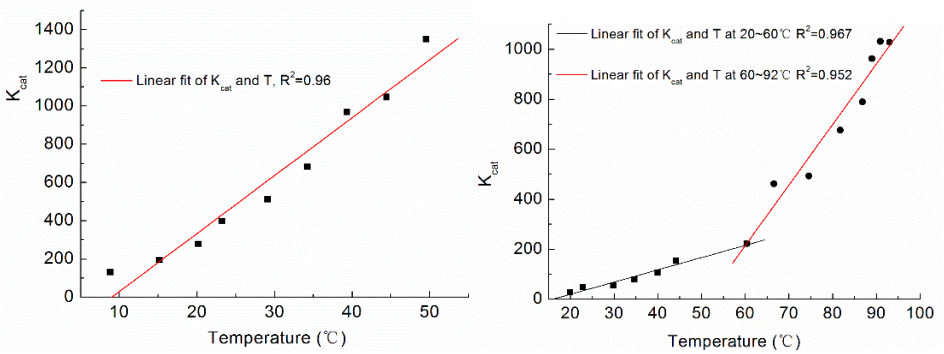

Fig.S2. Re-plot of published experimentally obtained correlation between temperature of the system and the turnover number (k_{cat}) of two enzymes mesoAdk(left) and thermoAdk(right), both of whose rate-limiting step is conformational change at substrate saturation condition, both support equation6. Dots and squares are experimental data. For thermoAdk, two phases can be observed, both of which show linear dependence on temperature. The k_{cat} and k_{open} of mesoAdk are 263s^{-1}, 286s^{-1} and the k_{cat} and k_{open} of thermoAdk are 30s^{-1}, 44s^{-1}, respectively, which indicate that conformational change accounts for probably 68%-92% of the rate limits at substrate saturation condition. Data from (Wolf-Watz, M., et al., *Linkage between dynamics and catalysis in a thermophilic-mesophilic enzyme pair.* Nature Structural & Molecular Biology, 2004. **11**(10): p. 945-949.)

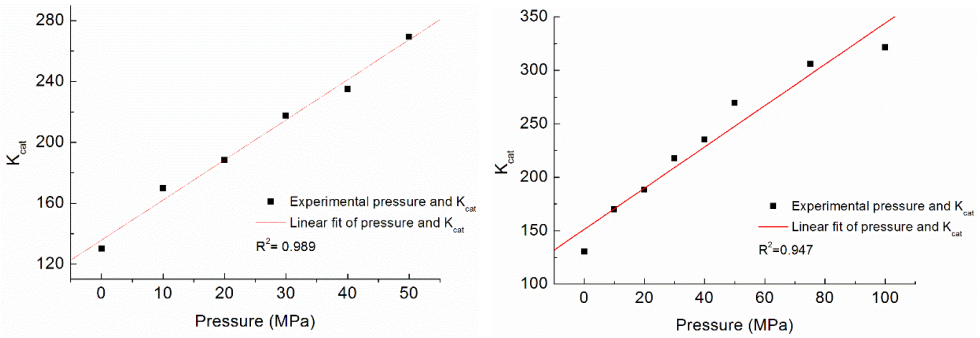

Fig.S3. Re-plot of published experimentally obtained correlation between pressure of the system and the turnover number (k_{cat}) of an enzyme PiezoAdk whose rate-limiting step is conformational change at substrate saturation condition, and this supports equation6. Squares are experimental data. Left, only data obtained within the pressure range of 0.1-50MPa is included; right, all data of pressure between 0.1-100MPa is included. K_{cat} values are the same under different pressures for another enzyme mesoAdk whose rate-limiting step is also conformational change at substrate saturation condition, this also supports the linear dependence of k_{conf} on pressure. Data from (Stiller, J.B., et al., *Probing the transition state in enzyme catalysis by high-pressure NMR dynamics.* Nature Catalysis, 2019. **2**(8): p. 726-734.)

Supplementary Discussion

History has come to a point where kinetic theories on enzymatic catalysis need to be systematically summarized and analyzed. With the development of modern science and technology, enzyme catalysis can be studied by a lot of novel technologies. Discrepancy between classical assumptions and emerging results begin to show. Biochemical reactions catalyzed by biological macromolecules are so diverse that a unified general principle is very difficult to find. This theoretical writing proposes the master equations based on the sequential events along the time axis of biological catalysis. A microscopic view of catalytic process is provided here, which tries to reconcile the discrepancy between traditional assumption and new results, and to link the kinetic experimental results with microscopic catalytic steps. Conceptual advancement is provided to connect the microscopic molecular behavior of enzymes to kinetic data obtained in experiment.

1. Explanations to questions on Figure S2, S3, equation6 and master equations

All elementary steps of catalysis are linked together by the master equations, including conformational change. Biophysical and biochemical conditions of the system like viscosity, temperature and pressure affect coefficient of conformational change k_{conf} (expressed by equation6), k_{conf} is related to k of the whole process by the master equations, and k equals to turnover number k_{cat} at substrate saturation conditions.

The relationship between the coefficient of conformational change and viscosity has been explored previously by experimental work including **(**Ansari, A., et al., *The role of solvent viscosity in the dynamics of protein conformational changes.* Science, 1992. **256**(5065): p. 1796-8.), which reveals the reverse linear correlation between the logarithm of them.

Plots in Figures S2 and S3 supports equation6, which describes the relationship between biophysical conditions of the system and k_{conf} of conformational change step.

The k_{cat} and k_{open} of mesoAdk are $263s^{-1}$, $286s^{-1}$, respectively, which indicate that conformational change accounts for 92% of the rate limits at substrate saturation condition. In this case, single turnover time $t=1/k=1/k_{cat}$ $=1/263s\approx0.0038s$. One kind of conformational change of mesoAdk is the 'open motion', which takes time $t_{open}=1/k_{open}$ $=1/286s\approx0.0035s$ within an averaged single turnover, then chemical conversion and 'close motion' and diffusion etc combined take time about 0.0038-0.0035s=0.0003s within an averaged single turnover. The reported 'close motion' takes time at the 10^{-4}s time scale. From this analysis, it is clear that 'open motion' takes the majority time of single turnover, the overall conformational change(open motion plus close motion) takes even more(>92%).

Three circumstances will be discussed to explain that the plots are supportive to equation6. ①If t_{conf} $(=t_{open}+t_{close})$ can be approximated by the value

of $t=1/k_{cat}$ over the whole tested temperature or pressure range, then the k_{cat} of Y axis of the plot roughly represents k_{conf}. Then the linear plots on Adk clearly support the relationship proposed by equation 6. ②If conformational change consistently accounts for the same ratio (eg. ~92%) of the single turnover time over the whole tested temperature or pressure range, then 100/92 times k_{cat} value of the plot roughly represents k_{conf}, the linear dependence proposed by equation 6 is proved and holds. ③Now, experiment shows that $k(k_{cat})$ is linearly dependent on T or P, for $1/t_{difu}$ or k_{difu}, the linear dependence has been proved by many, therefore, this experiment proves that the linear dependence of $1/(t_{conf}+t_{chem}+t_{prod})$, or $1/(1/k_{conf}+1/k_{chem}+1/k_{prod})$ on T or P holds. Even though reactant conformational change and product release conformational change are independent and separate, the equations on these two events all propose the linear dependence on T or P in highly similar formats, therefore, the two can be combined here into $t_{conf}(=t_{reactant\text{-}conf}+t_{prod\text{-}conf})$ or $k_{conf}[=1(1/k_{reactant\text{-}conf}+1/k_{prod\text{-}conf})]$. Then this experiment proves that the linear dependence of $1/(t_{conf}+t_{chem})$ or $1/(1/k_{conf}+1/k_{chem})$ on T or P holds. This can be largely in agreement with equation6, based on the catalytic features of Adk.

The author believes that a situation where conformational change corresponds to the major or large part of a single turnover like Adk is not necessarily required for the validity of equation 6. And equations 5,6,7,8,9,10,12 etc may be used either in combination with master equations or independently. And equation 6 may be applied to a variety of different situations like conformational change of single macromolecule or complex, binding induced or automatic conformational change, large scale or medium scale conformational change, etc.

2. Explanations to catalytic engineering and 'Increase in the values of factor c_{adjust} and factor A with improved k at either the same or slightly elevated temperature or pressure after enzyme engineering or directed evolution, with other conditions the same, both mean advancement or progress in enzyme activity elevation'. The catalytic coefficients k_{chem}, k_{conf}, k_{prod} are parameters that are independent of free substrate concentration or free enzyme concentration, in this way, the separable or independent factors that potentially affect the catalytic rate or velocity are preliminarily separated from each other. With contributions of physical conditions of the system to these catalytic coefficient parameters k_{chem}, k_{conf}, k_{prod} clearly defined by equation 6,7,8,9,10, etc, ultimately, factors or constants representing the properties of the enzyme at specific chemical conditions will be obtained, like the c_{adjust} factor of overall conformational-change process and factor A of chemical-conversion step, which is useful for application research and practices. Any one step of these tethering, conformational change, chemical conversion, and product release steps (or proton transfer step, if present) may be the one to optimize in catalysis engineering, directed evolution, pharmaceutical or medicinal research, signaling network modulation, metabolic pathway manipulation etc. Therefore, this perspective is potentially significant in academy, environment, agriculture, industry, and health related areas. The

microscopic steps of catalysis are the foundation to fine tune the catalytic character of enzyme, and provide a whole array of options for a variety of different purposes of catalytic engineering. The c_{adjust} factor of conformational-change process and factor A of chemical-conversion step may be the parameters to quantitively evaluate the optimization result at specific chemical conditions in enzyme engineering. Because overall activity elevation caused by increase in enzyme concentration or enzyme amount is usually not the goal of enzyme engineering or directed evolution. For instance, an increase of k_{chem}, k_{conf} at the same physical and chemical conditions of the system (inevitably with an increase of the values of c_{adjust} factor of conformational-change process and factor A of chemical-conversion step, respectively), or a huge increase of k_{chem}, k_{conf} at slightly elevated temperature or pressure(inevitably with an increase of the values of c_{adjust} factor of conformational-change process and factor A of chemical-conversion step, respectively) after enzyme engineering, both mean advancement or progress in enzyme activity elevation, because through any of these the catalytic efficiency represented by k can get improved. This is why the author states that the c_{adjust} of conformational change and A of chemical-conversion step may be the parameters to optimize for activity elevation in enzyme engineering.