**Chaper 1. Extended Methods**

*Statistical Analysis and Software Tools*

We used Excel and Python tools for regression analysis. We obtained the same results for the best-fit function, the determination coefficient, and the confidence interval for the exponent of the best-fit power function by utilizing corresponding tools in Excel and Python. For sensitivity analysis, we used the Python bootstrap re-sampling tool. Enzyme performance parameters and dissipation results from our dataset (see Dataset-S1-75) are not normally distributed. Thus, we used a non-parametric Mann-Whitney U test from Python to judge whether the association of specialized enzymes with higher dissipation is statistically significant. We employed Excel, Paint, and Python tools to construct figures. We created FORTRAN programs to simulate the transitions among steady states for all 75 enzyme-catalyzed reactions from our dataset in cases of a) standard state with all parameters derived or estimated from experiments (Figure 2 from the main text), and b) trade-off simulation (see below). The source codes and representative outputs for 58 enzyme-catalyzed reactions are available for download from Juretić (2025, Supplementary Material). The present contribution explores 17 additional reactions. Chapter 4 from the Supplementary Material of this contribution describes 17 home-made FORTRAN source codes and output Excel files we used to construct several figures from the main text. Those codes and files are available for download upon request. Figures 2, 3, 4, 6, and 7 assembled selected data from the present contribution and Juretić (2025).

*Equations for kcat, kcat/KM, and the Dissipation Function*

We used well-known equations (Heinrich et al., 1991; Wilhelm et al., 1994) for forward (S → P) Michaelis constants and maximal activities in the case of two, three, and four-state cyclic kinetic mechanisms (see Figure 1 from the main text). Substrate and product concentrations are mostly not explicitly present in the following equations because they were multiplied with the second-order rate constants. For the two-state reversible cycle (Figure 1a):

**=** and (1)

For the three-state cyclic kinetic mechanism (Figure 1b):

and (2)

For the four-state cyclic kinetic mechanism (Toney, 2013) (Figure 1c):

(3)

and

(4)

All of these equations are strictly valid only for uni-uni enzymatic reactions. However, Karamitros et al. (2024) used the same four-state parameters, kcat and KM (Eqs. 3 and 4), when one substrate enters the reaction and two products exit it. These authors utilized the symbol k1 for the second-order substrate binding constant, which is k1/[S] in our notation. Thus, we also used those equations for the four-state catalysis mechanism of carbonic anhydrases, soluble inorganic pyrophosphatase, and kynureninases when two products enter or exit the reaction. We often used the maximal reaction rates and the Michaelis constant in the forward and reverse direction for two-state reversible kinetic models to find all four microscopic rate constants.

With all of the microscopic rate constants known, we followed Terrel L. Hill (1977) in finding the expressions for the single cycle forces and fluxes in a steady state. The two-state expressions for net reaction flux J and the thermodynamic force X, are respectively:

and (5)

where K = K1∙K2 is the equilibrium constant. Three-state expressions are

(6)

and

(7)

with K = K1∙K2∙K3.

Four-state expressions are

(8)

with

and   
 (9)

where K = K1∙K2∙K3∙K4.

The dissipation function φ is then the J∙X product in each case. We used the convention of treating all microscopic rate constants equally as first-order constants. The identity and concentration of substrate and product or products can be found in the Dataset-S1-75.

*Introducing Normal Noise in Microscopic Rate Constants*

In simulations, we multiplied each of the observed or estimated forward rate constant ki with the Box-Muller transform (Box and Muller, 1958),

(10)

where *s1* and *s2* are random numbers chosen from the unit interval (0, 1) by the standard FORTRAN generator *random\_number*. The shift +1.0 gives prominence to positive numbers for modified rate constants. Since rate constants ki > 0, we used only positive gi values for simulations. Simulations had 10000 to 30000 steps, resulting in the same number of rows in the program's output. Rows with negative random numbers are replaced with the first previous row with a positive random number. In trade-off variations, we allowed for the compensatory changes in the equilibrium constants for the enzyme-substrate association and the last reaction step of the enzyme-product dissociation. We also kept the total force constant X in these simulations.  We did not derive the general proof that the maximum in the entropy production can always be found, but we did find that maximum in all 75 reactions from our dataset (Dataset-S1-75). We had to increase the substrate concentration to reach the maximum in several reactions (see Table 2 of Juretić, 2025). Once the steady state with maximum dissipation is found, all other (optimal) parameters can be compared with their estimated or observed values. Either catalytic constant or enzyme efficiency is higher for the maximal dissipation state. The maximal kcat/KM for trade-off variations is associated with similar or smaller dissipation from the observed value.

*The Dataset Collection*

The complete set of microscopic kinetic constants in forward and reverse directions have been occasionally measured or estimated for some enzymes with simple uni-uni kinetic mechanisms. To our knowledge, there was never a systematic effort to collect a complete set of ki values for as many enzymes as possible. Moreover, the interest in publishing all ki values for studied enzymes waned through decades, possibly because kcat and KM kinetic constants (macroscopic kinetic data) were considered sufficient for the kinetic analysis. Free energy profile determination requires the knowledge of all ki values. However, free energy profile construction remains challenging. It involves combining complex experimental techniques with sophisticated computational optimizations (Toney, 2013).

To collect our database, the Dataset-S1-75, we first used many different keywords and phrases in the Google Scholar or PubMed search combined with the word "enzyme." Examples are "characterization," "simulation," "catalytic properties," "catalytic rate," "rate constants," "microscopic rate constants," "energy profile," "kinetic analysis," "kinetics of," "catalytic properties," "catalytic steps," "catalytic cycle," "kinetic and thermodynamic," "evolution of," and others. After searching through hundreds or thousands of papers, none of the keywords or phrases helped find more than two to four articles cited in the Dataset-S1-75 (Behravan et al., 1990; Toney, 2019; Juretić et al., 2019; Zyryanov, 2004; McIntyre et al., 1989; Liu et al., 2005; Das et al., 2013; Käpylä et al., 1995; Cooperman et al., 1992; St Maurice and Bearne 2002; Darvey et al., 1975; Zawrotny and Pollack, 1994; Hwang and Hyeon, 2017; Fraser et al., 1999; Mattei et al., 1999; Holliday et al., 2015; Mutaguchi et al., 2013; Kim et al., 2010; Pozo-Dengra et al., 2009; Mehboob et al., 2009; Watanabe et al., 2015; Kato et al., 2012; Liu et al., 2021; Miyamoto et al., 2022; Yaneva et al., 2012; Gogami et al., 2010; Christenson et al., 2003; Converti et al., 1998; Gaily et al., 2013). Searching for some enzyme classes, such as mutases, epimerases, isomerases, and racemases, can improve the search strategy due to the simplicity of their catalytic mechanism and the low total number of ki constants (see Figure 1 from the main text). The focus on two-state reaction cycles (Figure 1a) also helped get the desired results due to the possibility of extracting the complete set of four ki constants from Vm and KM parameters determined in both directions (Darvey et al., 1975). A good strategy was to examine publications of experts who appreciated the insight that complete kinetic characterization offered into enzyme evolution and the nature of their catalytic mechanism. To mention just a few of them, W. John Albery and Jeremy R. Knowles (1976), Michel D. Toney (2013), and Kenneth A. Johnson (2019) took the challenge to extract information about enzymatic free energy profiles from the determination of microscopic rate constants. The BRENDA enzyme database (Schomburg et al., 2017)(Braunschweig enzyme database) is a rich source for enzymatic reactions. There is no obvious way to search in BRENDA for all instances when a complete set of microscopic rate constants has been determined or estimated. None of the items from Dataset-S1-75 was first found in BRENDA or other databases. Data retrieval is still an open issue with the STRENDA database (Swainston et al., 2018). Only 35 research papers were the origin for the parameters we collected in the Dataset-S1-75 Excel table. Five papers (Knowles and Albery, 1977; Liu et al., 1990; Liu et al., 2005; Hoffman et al., 2009; Penkler et al., 2015) were used in this contribution for extracting parameters but not in our previous paper (Juretić, 2025).

We did not intend to collect the required data exclusively for soluble proteins. Our Database-S1-75 has one example of the enzyme "walking" in one dimension (kinesin-1), one example of the membrane-associated enzyme (F1-ATP synthase), and several examples of immobilized enzymes. We considered them equivalent to soluble proteins for our analysis. Therefore, we also used measured microscopic rate constants to calculate all parameters for such cases. Membrane proteins likely succumb to the same thermodynamic drive as soluble proteins. Increased complexity in calculating performance parameters and overall dissipation for membrane enzymes influenced our choice to consider a similar analysis for integral membrane enzymes beyond the present manuscript's scope.