**Supplementary Materials**

**Factors determining the enzyme catalytic power caused by noncovalent interactions: charge alterations in enzyme active sites**

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**Figure S1. Definition of H-bonding capability and the accuracy of using H-bonding capability to quantify the effects of noncovalent interactions on free energy barriers (ΔG‡s) of reactions.** The red symbol R represents a polar atom; the grey symbol R represents the depolarized R; Sub represents a non-polar group, R‡ is the transition state of R. All free energy changes (ΔGs) are the standard free energy changes caused by the change in noncovalent interactions.

(**A**)  *Definition of H-bonding capability:* The H-bonding capability of a polar atom R (HR) or a molecule (HM) is the water to nonpolar solvent phase transfer free energy contributed by the polarity of the atom or the molecule.([Chen et al., 2019](#_ENREF_2); [Chen et al., 2016](#_ENREF_3)) The thermodynamic cycle shows that the H-bonding capability of the molecule SubR can be calculated as

HM = ΔG1 –ΔG2 + ΔG3 = ΔG1 –ΔG2 (S1)

where ΔG1 represents the ΔG required to transfer the molecule from water to hexadecane and ΔG2 represents the ΔG required to transfer the depolarized molecule from water to hexadecane. Because neither the molecule nor depolarized molecule has electrostatic interactions with non-polar solvent, ΔG3 is zero. Thus, HM is the ΔG required to transfer the molecule from water to hexadecane minus the ΔG required to transfer the depolarized molecule from water to hexadecane. If a molecule contains only one polar atom R, the H-bonding capability of R (HR) equals HM. The HM for water is 28.07kJ/mol. Because a water molecule has two hydrogen atoms and two lone pairs of electrons and the H-bond strengths for the hydrogen atoms and lone pairs of electrons are identical. Thus, the H-bonding capabilities for the hydrogen atom and the lone pair of electrons in water are 7.02 kJ/mol.

*(****B****) Accuracy of using H-bonding capability to quantify the effects of noncovalent interactions on* ΔG‡*s.* Figure S3B is the thermodynamic cycle for quantifying the effect of the noncovalent interaction between a substrate atom R and bulk water on the ΔG‡ of the reaction in aqueous solution. This thermodynamic cycle indicates that the difference between the ΔG‡ of the reaction in bulk water (ΔG‡aqu) and in nonpolar solvent (ΔG‡nonpol) equals the difference between the water to nonpolar solvent phase-transfer energy of the substrate in the GS and in the TS(ΔG3 –ΔG4)，which in turn equals the different between the H-bonding capability of R (HR) and the H-bonding capability of R‡ (HR‡)

ΔG‡aqu – ΔG‡nonpol =ΔG3 –ΔG4 = HR – HR‡ (S2)

Equation (S2) indicates that the effect of the electrostatic interaction between a substrate atom and bulk water on the ΔG‡ of the reaction in aqueous solution corresponds exactly to the change in the H-bonding capability of the atom between the GS and the TS. In a previous study, we also demonstrated that H-bonding capability can be used to accurately quantifying the effect of the noncovalent interactions in other environments (e.g. enzyme active sites) on the ΔG‡s of reactions. Relationships derived from thermodynamic cycles are absolutely correct. Thus, equation (S2) is absolutely correct and H-bonding capability is an ideal parameter for accurately quantifying the effects of noncovalent interactions on ΔG‡s of reactions.

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**Figure S2. Electronic effects on the electron densities and H-bonding capabilities of polar atoms*:***The electron density and H-bonding capability of an atom depends on electronic effects of the atom’s connections, which include resonance and inductive effects. (**A**) Resonance effects where electron redistribution of X, Y, and H is caused by delocalization through interconnected π-bonds. (**B**) Inductive effects where redistribution of electron density of Y and H occurs through σ bonds. (**C**) Plot of the logarithm of H-bonding capability for the polar hydrogen atom from a substituted phenol against the Hammett constant of the substituent([Hansch et al., 1991](#_ENREF_4)) for demonstrating the resonance effects on H-bonding capabilities reveals a linear trend with a satisfactory correlation coefficient. (**D**) The H-bonding capabilities of the H-bond donors in OH groups connecting to groups with different electronic effects. The data shown in Figures S2C&D indicate that electron withdrawing groups increase the H-bonding capabilities of H-bond donors *via* resonance and inductive effects and electron releasing groups decrease the positive charge densities and H-bonding capabilities of H-bond donors.

*Relationship between H-bonding capability and the charge density of a polar atom:* Figures S2A&B indicate that electronic effects on the electron densities and H-bonding capabilities of polar atoms are the same. Thus, polar atoms with higher charge density will have higher H-bonding capabilities. This conclusion is supported by the data shown in Figures S2C&D.

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**Figure S3. Comparing the positive charge densities of the hydrogen atoms from Tyr16 and Asp103 with those of the hydrogen atoms from water**. A lone pair of electrons of the oxygen atom (colored red) from the OH group of Asp103 is delocalized to the C=O group of Asp103. A lone pair of electron of the oxygen atom (colored red) from the OH group of Tyr16 is delocalized to aromatic ring of Tyr16. As a result, the negative charge densities of the oxygen atoms are less than that of the oxygen atom of water. The positive charge densities of polar hydrogen atoms (colored blue) from the OH groups of Asp103 andTyr16 are larger than the positive charge densities of the hydrogen atoms of water.

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**Figure S4. Catalysis by electrostatic preorganization can be explained by the charge alteration mechanism.** Figure S4A shows the H-bonds in the electron-accepting center of the KSI-catalyzed isomerization of 5-AND. The corresponding unstrained H-bonds in solution are shown in Figure 4B. The preorganized H-bonds in Figure S4A are close to the optimal H-bond geometry and are stronger than the corresponding unrestrained H-bonds in Figure S4B. Thus, the substrate oxygen atom in Figure S4A is in an environment with larger positive charge density than the substrate oxygen atom in Figure S4B. Because the electrostatic preorganization in Figure S4A enhances the positive charge density in the electron-accepting center, the catalysis by this electrostatic preorganization supports the charge alteration mechanism developed in this study.

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**Figure S5. The reason why the His119 in wild-type CAII is neutral and the His119 in E117Q CAII is negatively charged**. We have demonstrated this in a previous study.([Chen et al., 2018](#_ENREF_1)) Because it is not easy to understand why the His119 in E117Q CAII is negatively charged, we further illustrate the reason in this study. (**A**) The distance between the oxygen atom (or the nitrogen atom) of residue 117 and the nitrogen atom of His119 in wild type CAII or in E117Q CAII ranges from 2.6 to 2.8Å, which is obtained from the crystal structures of wild type CAII, E117Q CAII, and their complexes with inhibitors. This distance indicates that only one hydrogen atom exists between X and the nitrogen atom of His119. Thus, either residue 117 or His119 must be negatively charged. (**B**) Because the negatively charged carboxyl group is more stable than the negatively charged His119, His119 in wild-type CAII is neutral. (**C**) Because the pKa of the imidazole group in histidine (~6.3) is much lower than that of amide group (~18.0), the fraction of the negatively charged amide group is close to zero (1/10(18.0-6.3) ≈ 0) and the negative charged His119 is more stable than the negatively amide group in E117Q CAII. Thus, His119 in E117Q CAII is negatively charged.

**References**

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