Electronic Supplementary Information

Key Difference between Transition State Stabilization and Ground State Destabilization: Increasing Atomic Charge Densities Before or During Enzyme-Substrate Binding

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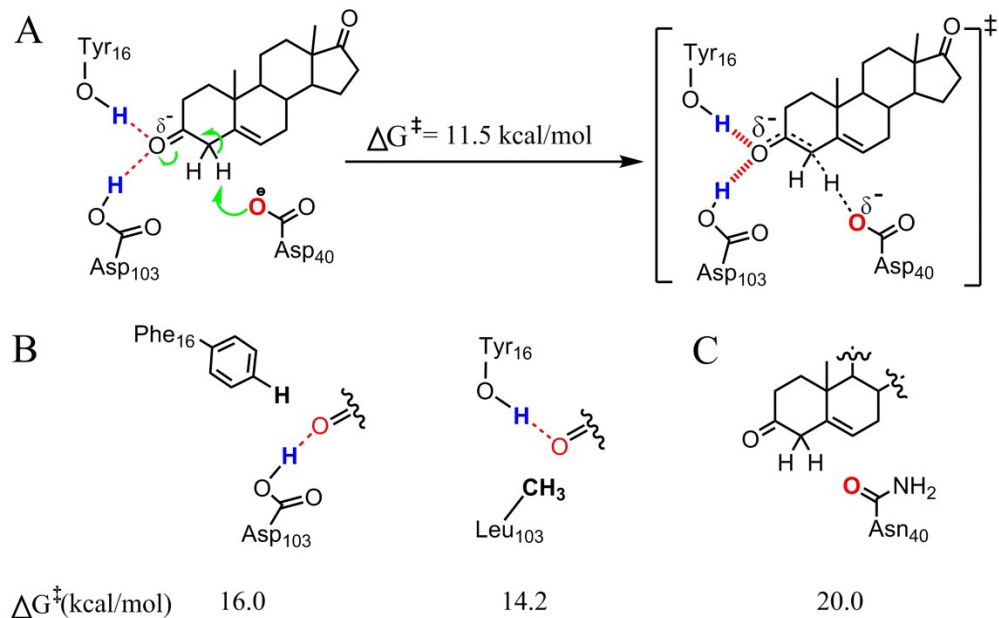**Supplementary Figures**

Supplementary Figures

Figure S1. Enzyme-substrate interactions supporting the TS stabilization and supporting GS destabilization mechanisms. (**A**) The ketosteroid isomerase (KSI) catalyzed the isomerization of 5-androstene-3,17-dione (5-AND). The free energy barrier ($\Delta G^{\ddagger}$) of this enzymatic reaction is 11.5 kcal/mol.[1] (**B**) Electrostatic TS stabilization. The $\Delta G^{\ddagger}$s of the reaction catalysed by KSI Y16F mutant and the reaction catalysed by KSI Y16L mutant are 16.0 kcal/mol and 14.2 kcal/mol.[1] Thus, the H-bonds of substrate oxygen atom with Tyr16 and with Asp103 reduce the $\Delta G^{\ddagger}$ of the reaction by 4.5 kcal/mol and 2.7 kcal/mol, respectively, supporting the electrostatic TS stabilization mechanism. (**C**) GS destabilization. The $\Delta G^{\ddagger}$ of the reaction catalysed by KSI D40N mutant is 20.0 kcal/mol.[1] The binding affinity of the substrate to KSI D40N mutant is stronger than the binding affinity of the substrate to wild type KSI,[2] supporting that the GS destabilization of the negatively charged COO$^-$ group from Asp40 provides important contribution to the catalysis of KSI.
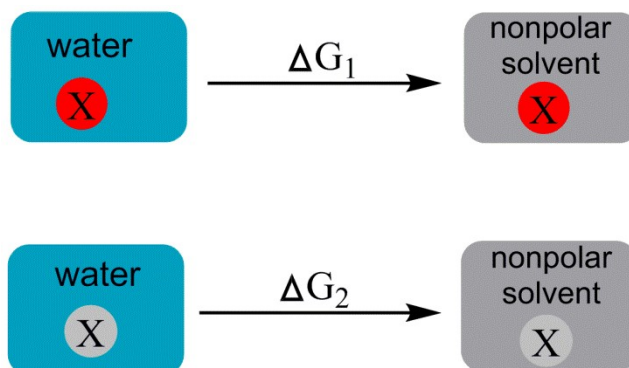
Figure S2. H-bonding capability and its relationship with atomic charge density. The red symbol X represents a polar atom or a molecule; the grey symbol X represents depolarized X. The H-bonding capability of the molecule X (H_X) is the water to nonpolar solvent phase transfer free energy contributed by the polarity of X. It is the difference between the free energy for transferring X from water to nonpolar solvent (ΔG_1) minus the free energy for transferring depolarized X from water to nonpolar solvent (ΔG_2).

$$H_X = \Delta G_1 - \Delta G_2 \qquad , \qquad\qquad\qquad\qquad (S1)$$

If X is nonpolar, ΔG_1 equals ΔG_2 and H_X is zero. Increase the charge density of X, the electrostatic interaction between X and water becomes stronger and the free energy for transferring X from water to nonpolar solvent, ΔG_1, becomes more positive. Thus, an atom (or a molecule) with higher charge density will have larger H-bonding capability.
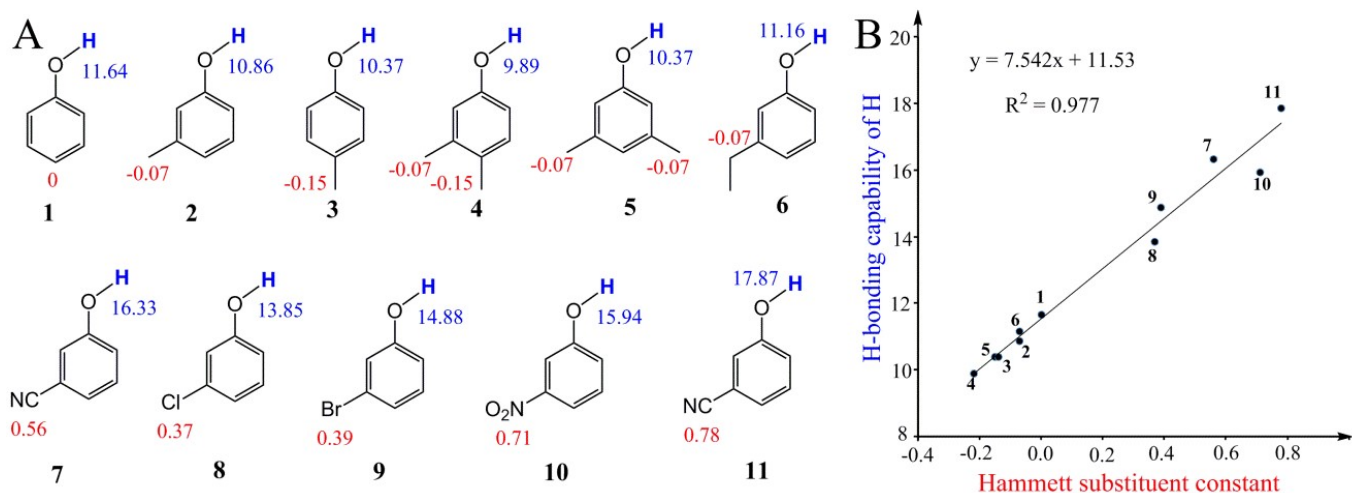
Figure S3: Relationship between the H-bonding capabilities of the polar hydrogen atoms of substituted phenols and the positive charge densities of the atoms. (**A**) The H-bonding capabilities (colored blue) of the hydrogen atoms in a serious of substituted phenols and the Hammett constants (colored red) of the substituents. The H-bonding capabilities of the hydrogen atoms are calculated from the experimental $logP_{16}$ (logarithm of the hexadecane–water partition coefficient) and $logP_{oct}$ (logarithm of the n-octanol–water partition coefficient) values of the substituted phenols (see Methods). (**B**) Regression of the H-bonding capability of the polar hydrogen atom in a substituted phenol against the sum of Hammett constants of the substituents in the substituted phenol. The regression result indicates that there is a strong positive linear association between the H-bonding capability of the hydrogen atom and the sum of Hammett constants of the substituents. Also, substituents with larger positive Hammett constants have stronger electron-withdrawing abilities and increase the positive charge densities of the hydrogen atoms to a larger extent, indicating that larger positive Hammett constant corresponds to higher positive charge density. Based on the relationship between the H-bonding capability and Hammett constant and the relationship between Hammett constant and charge density, we conclude that an atom with higher H-bonding capability will have high charge density.
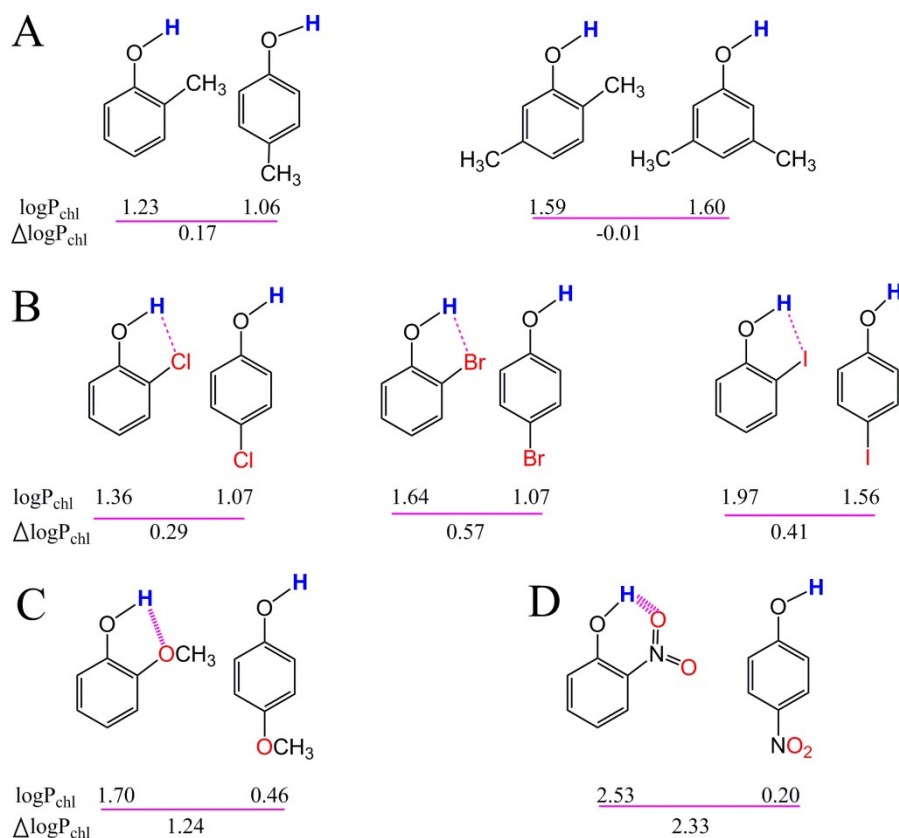
Figure S4. Effects of the electrostatic interactions of polar atoms on the charge densities of the atoms. To determine the effects of electrostatic interactions on atomic charge densities, we compared the water to chloroform partition coefficients ($logP_{chl}$) for a series of 2-substituted phenols and the $logP_{chl}$ values of structurally similar compounds with the substitutes in the 4- and/or 3- positions. Because an atom with larger H-bonding capability will have higher charge density (see Figures S2 and S3), we can determine the effects of the electrostatic interactions on atomic charge densities by investigating the effects of intramolecular H-bonds on the $logP_{chl}$ values of the compounds containing the intramolecular H-bonds.

H-bonding abilities of the H-bond acceptors in the 2-position: For the compounds in (A), the atoms in the 2-position are nonpolar and are not H-bond acceptors. For the compounds in (B), the substitutes (Cl, Br and I) are weak H-bond acceptors. For the compound in (C), the oxygen atom (colored red) is a

stronger H-bond acceptor than the halide atoms in (B). Because the electrons of this oxygen atom are delocalized to the aromatic ring, the oxygen atom is a weaker H-bond acceptor than the oxygen atom in (D). Thus, the relative H-bonding abilities of the H-bond acceptors in the 2-position are (D) > (C) > (B) > (A).

Strengths of the intramolecular H-bonds. For the compounds in (A), the compounds cannot form intramolecular H-bonds. As the H-bond donors for all compounds are almost the same, the relative strengths of the intramolecular H-bonds are (D) > (C) > (B) > (A) = 0.

Effects of the intramolecular H-bonds on logP$_{chl}$ values of the compounds with intramolecular H-bonds. For the compounds in (A), the logP$_{chl}$ values for 2-substituted phenols are close to the logP$_{chl}$ values of structurally similar compounds with the substitutes in the 4- or 3-position. For the compounds in (B), the logP$_{chl}$ values for 2-substituted phenols are about 0.42 ± 0.10 log unit lower than the logP$_{chl}$ values of structurally similar compounds with the substitutes in the 4-position. For the compounds in (C) and (D), the logP$_{chl}$ values for 2-substituted phenols are 1.24 and 2.33 log units lower than the logP$_{chl}$ values of structurally similar compounds with the substitutes in the 4-position. The results indicate that intramolecular H-bonds reduce the logP$_{chl}$ values of the compounds containing the intramolecular H-bonds. Stronger intramolecular H-bond reduces logP$_{chl}$ to a larger extent. Therefore, electrostatic interactions of polar atoms reduce the charge densities of the atoms, with stronger electrostatic interactions reducing charge densities to a larger extent.
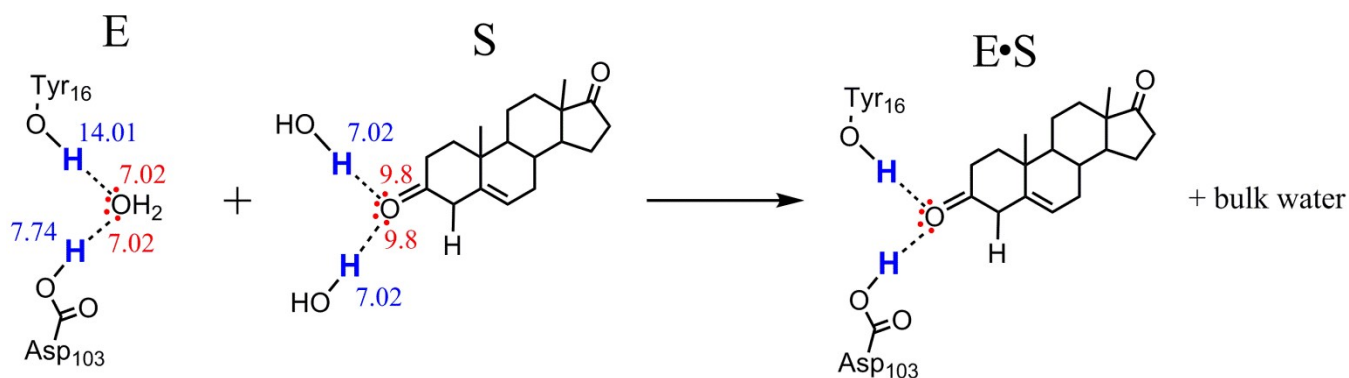
Figure S5: Binding free energy contributed by the electrostatic interactions in the KSI-catalyzed isomerization of 5-AND. The illustrations colored in blue or in red represent the H-bond capabilities of nearby polar atoms (in kJ mol^{-1}). The formation of the electrostatic interactions of the substrate oxygen atom is a competitive H-bond paring process as shown in this figure. The free energy change of this process, which is the binding free energy of 5-AND to KSI, can be calculated from the model for calculating the free energy change of a competitive H-bond paring process as reported in a previous study.[3] Thus, the binding free energy contributed by the electrostatic interactions in the KSI-catalyzed isomerization of 5-AND is

$$\Delta G^{HB}_{bind\_GS} = -\Sigma(H_E - H_{wat})(H_R - H_{wat})/H_{wat}$$

$$= -(14.01 - 7.02)(9.8-7.02)/7.02 - (7.74 - 7.02)(9.8-7.02)/7.02$$

$$= -3.05\text{kJ mol}^{-1} = -0.73\text{kcal mol}^{-1}. \tag{S2}$$

This result indicates that H-bonds stabilize the GS of the reaction.

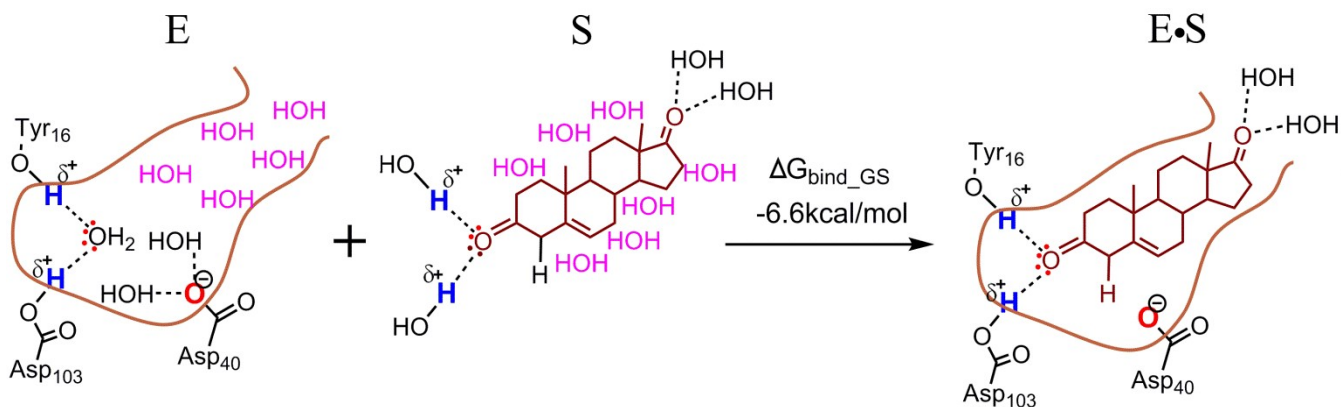**Figure S6. Large effect of the interactions not affecting $\Delta G^{\ddagger}$ on the binding affinity of 5-AND to KSI.** The interactions between 5-AND and KSI include the interactions affecting the $\Delta G^{\ddagger}$ and he interactions not affecting the $\Delta G^{\ddagger}$. The interactions affecting the $\Delta G^{\ddagger}$ include the H-bond interactions of the oxygen atom of 5-AND, which stabilizes the GS slightly (0.73kcal mol^{-1}, Figure S5), and the interaction between the nonpolar substrate α-H and the negatively charged oxygen atom from Asp40, which destabilizes the GS largely. Based on reported experimental data, the binding free energy of 5-AND to KSI is about -6.6kcal/mol.[4] This GS stabilization results from the enzyme-substrate interactions not affecting $\Delta G^{\ddagger}$.

In the KSI-catalyzed isomerization of 5-AND, the enzyme-substrate interactions not affecting $\Delta G^{\ddagger}$ are mainly hydrophobic interactions (transferring water molecules from non-polar surface area of 5-AND and enzyme active site to bulk water). The free energy contributed by the hydrophobic interactions (ΔG_{HP}) can be calculated from the change of the surface accessible surface area ($\Delta SASA$) during the binding process based on the following model[3]:

$$\Delta G_{HP} = -(0.19124 \times \Delta SASA - 0.4824 \times flex - 3.116) \text{ (kJ/mol)} \tag{S11}$$

The term flex is the number of the flexible bonds that are fixed during the binding process. The total $\Delta SASA$ is the sum of the $\Delta SASA$ for KSI and the $\Delta SASA$ for 5-AND and is about 526Å^2, which is calculated based on the crystal structures of KSI mutant-steroid substrate mimic complex (pdb code:

1E3R[5]). The calculated ΔG_{HP} is about -22.5 kcal mol^{-1}. Also, some polar atoms have electrostatic interactions with water before binding and the interactions do not exist after binding. Thus, there is an unfavorable free energy relevant to the desolvation of the polar atoms (ΔG_{desol}). Based on the H-bonding capabilities and the surface accessible surface areas of the polar atoms, we estimated the ΔG_{desol} is about 5.2 kcal mol^{-1}. Thus, the contribution of the interactions not affecting $\Delta G^{\ddagger}$ on the binding free energy of 5-AND is about -17.3kcal mol^{-1}, indicating that the interactions not affecting $\Delta G^{\ddagger}$ stabilize the GS of the KSI-catalyzed isomerization of 5-AND largely. As the interactions do not affect $\Delta G^{\ddagger}$, they also stabilize the TS of the reaction largely.
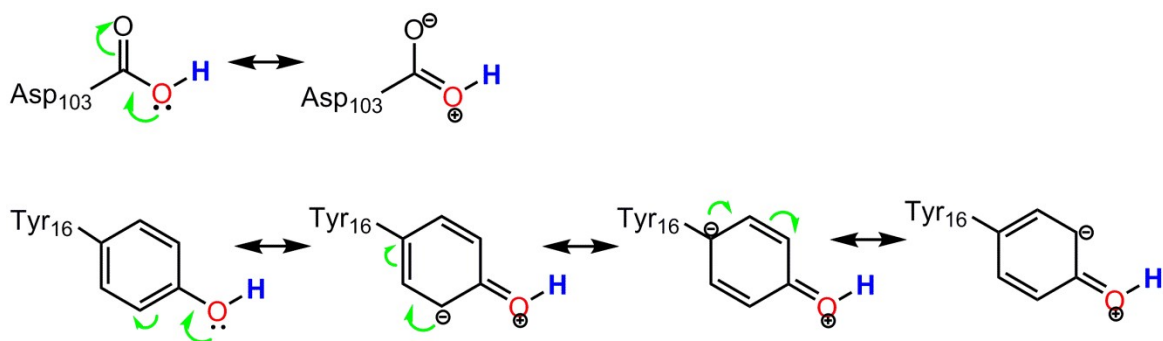
Figure S7. Higher positive charge densities of the polar hydrogen atoms in the oxygen hole of KSI as compared to the hydrogen atoms of water. A lone pair electrons of the oxygen atom (colored red) from the OH group of Asp103 is delocalized to the C=O group of Asp103. A lone pair electron of the oxygen atom (colored red) from the OH group of Tyr16 is delocalized to aromatic ring of Tyr16. As a result, the electron densities of the oxygen atoms are lower than the electron density of the oxygen atom of water, which enhances the positive charge densities of the polar hydrogen atoms (colored blue) from the OH groups of Asp103 andTyr16. As a result, the polar hydrogen atoms in the oxygen hole of KSI have higher positive charge densities than the hydrogen atom of water.
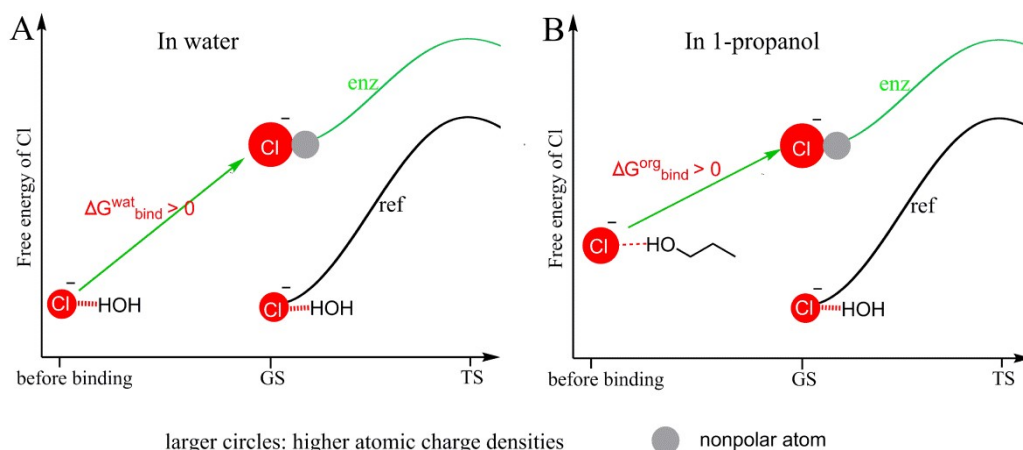
Figure S8. Desolvation and the charge density alteration of Cl⁻ in the halogenation of organic compounds catalyzed by haloperoxidase in water (A) and in 1-propanol (B). Before the enzyme-substrate binding, the Cl⁻ in 1-propanol interacts with the polar hydrogen atom of 1-propanol, but the interaction is weaker than the corresponding interaction in water. Thus, the Cl⁻ in 1-propanol is less solvated than the Cl⁻ in water and is partially desolvated before the enzyme-substrate binding. The Cl⁻ in 1-propanol has higher charge density than the Cl⁻ in water, but has lower charge density than the Cl⁻ in nonpolar environment. In the binding process, the Cl⁻ moves from solvent (1-propanol or water) to the nonpolar environment of enzymatic active site and is desolvated. The desolvation energy in 1-propanol (ΔG^{org}_{bind}, Figure S8B) is less than the desolvation energy in water (ΔG^{wat}_{bind}). The charge density of the Cl⁻ in the GS of the enzymatic reaction performed 1-propanol is the same as that in the enzymatic reaction performed in water (assume 1-propanol does not change the structure of the enzyme). Thus, the Cl⁻ in 1-propanol is partially desolvated before the enzyme-substrate binding and is partially desolvated during the enzyme-substrate binding.
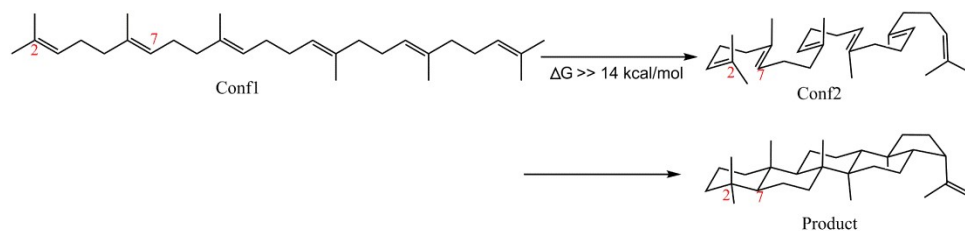
Figure 9: Polycyclization of polyisoprene for demonstrating the catalysis by facilitating the conformation changes of substrates in the enzyme-substrate binding processes. In the polycyclization of polyisoprene, polyisoprene in its most stable linear conformation (**c**onf1) cannot take the reaction because the atoms (*e.g.* atoms 2 and 7 in conf1) for forming chemical bonds are far from each other. It must change to the productive precyclic conformation (conf2) so that chemical bonds can form. However, conf2 is much less stable than conf1 (14 kcal at 328K for monocycle[6]). The concentration of conf2 is much lower than the concentration of conf1 ([conf2]/[conf1] $<<$ 10^{-9}) in aqueous solution. In the polycyclization of polyisoprene catalyzed by terpene cyclase, the substrate in the enzyme-substrate complex is in conf2. [conf2] is close to the concentration of the enzyme when the enzyme-substrate binding is strong. The release of ordered water molecules in the enzyme-active site and nonpolar surface of the substrate is the main approach to provide energy for the strong enzyme-substrate binding. Thus, in this example, the enzyme catalyzes the reaction by facilitating the conformation change of the substrate in the enzyme-substrate binding process. The entropy change caused by the release of ordered water molecules is the main origin of the enzyme catalysis.

References

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