Misunderstanding the Preorganization Concept can lead to Confusions about the

Origin of Enzyme Catalysis

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EVB Simulations

We calculated the activation energies of systems **I-V** given in the main text using our empirical valence bond (EVB)[1-3] and free energy perturbation/umbrella sampling (FEP/US) approach.[4] The calculations were performed using MOLARIS software with the ENZYMIX force field.[5] The EVB method has been widely used by our group and others and below we provide a concise description of the method. The system is divided into the EVB region and rest of the protein. The classical forcefield ENZYMIX is used to simulate the remaining protein whereas the EVB region is treated using a quantum empirical valence bond forcefield. The reaction is simulated from the reactant state to the product state which represent the two diabatic states. The ground state is obtained by mixing the two diabatic states.

In the EVB forcefield, the bonded atoms are defined using a Morse potential. For a two diabatic state system, ground state potential is:

$$Eg = c_1^2 \varepsilon_1 + c_2^2 \varepsilon_2 + 2(c_1^2 c_2^2)^{1/2} H_{12} \tag{S1}$$

ε_1 and ε_2 are the potential of the two diabatic states.

The coefficients are determined by diagonalizing the matrix,

$$H = \begin{pmatrix} \varepsilon_1 & H_{12} \\ H_{12} & \varepsilon_2 \end{pmatrix} \tag{S2}$$

The mixing term H_{12} is:

$$H_{12} = A exp\{-\mu R\} \tag{S3}$$

where, A and μ are empirical constants that are calibrated using experimental free energy. These parameters are considered to be same in the protein and the reference state. To simulate the bond formation/breaking between two EVB states, simulations are carried out on a mapping potential which is as follows:

$$\varepsilon_m = (1 - \lambda_m)\varepsilon_1 + \lambda_m \varepsilon_2 \qquad (0 \leq \lambda_m \leq 1) \tag{S4}$$

where, λ_m is a parameter that is changed from 0 to 1 in N+1 windows. The Free Energy perturbation (FEP) is used to calculate the free energy change between two consecutive steps:

$$\Delta G_{m \to m+1} = -\beta^{-1} ln \langle e^{\{-\beta[\varepsilon_m(\lambda_{m+1}) - \varepsilon_m(\lambda_m)]\}} \rangle_m \tag{S5}$$

$< >_m$ represents an average over different configurations when system moves on the ε_m potential.

The activation free energy is calculated using the free energy functional that represents the adiabatic ground state surface:

$$\Delta g(x') = \sum_{m=0}^{i-1} \Delta G_{m \to m+1} - \beta^{-1} ln \left\langle \delta(x - x') e^{\{-\beta[E_g - \varepsilon_m(\lambda_i)]\}} \right\rangle_{\varepsilon_m} \qquad (S6)$$

where, $\sum_{m=0}^{i-1} \Delta G_{m \to m+1}$ = Free energy difference between the first and i^{th} mapping potential, E_g = Energy of the ground state and δ = Dirac delta function and the inner broken brackets.

For the current study, we considered a similar mechanism as given in Ref. [6]. The EVB surface is generated using a two state diabatic model, where the two states represent the reactant state (RS) and the transition state (TS). The method of representing the TS as a minimum has been previously used by our group and is described in detail in Ref. [7]. The RS and TS were first subjected to geometry optimization at the M06-2X/6-311+G** level of theory using Gaussian09 software.[8] The geometric parameters obtained using the quantum approach were used to define our EVB parameters. The electrostatic potential charges (ESP) charges for the two diabatic states that represent the RS and TS were also calculated using the M06-2X level of theory at the 6-311+G** basis set. The EVB region was treated as the center of the system which was immersed in an 18Å water sphere using the surface constrained all atom solvent (SCAAS) model.[9] Local reaction field (LRF) was used to treat the long range effects.[10] The different systems were first relaxed by heating the system from 30K to 300K for 100 ps. Three different starting structures were generated from this relaxation and were used for the FEP simulation which involved 31 frames where each frame was simulated for 20 ps with a time step of 1 fs. The atom numbering scheme is provided in Figure S1 and the EVB parameters are given in Tables S1a-g.
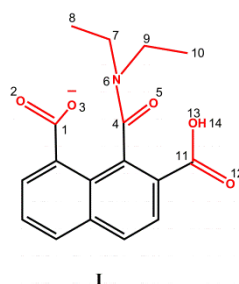

Figure S1. Atom numbering scheme for system **I** for EVB region which is shown in red

Table S1. EVB parameters

a- Atomic Charges for the Reactant and Transition State

Atom	Atom Name	RS	TS
1	C	0.970	1.075
2	O	-0.922	-0.791
3	O	-0.865	-0.801
4	C	0.307	1.031
5	O	-0.642	-0.911
6	N	0.101	-0.295
7	C	0.089	0.064
8	C	-0.346	-0.278
9	C	0.174	0.195
10	C	-0.310	-0.419
11	C	0.795	0.885
12	O	-0.663	-0.722
13	O	-0.599	-0.723
14	H	0.489	0.417
15	H	0.058	0.062
16	H	0.033	0.059
17	H	0.030	0.034
18	H	-0.002	0.013
19	H	0.111	0.090
20	H	0.105	0.074
21	H	0.077	0.066
22	H	0.091	0.133
23	H	0.072	0.113
24	H	0.083	0.110

b- Morse Bond Parameters; $\delta M(b) = D_M (1 - e^{-\mu(b-b_0)})^2$

Bond Type	D_M	b_0	μ
C1-O2	93.0	1.250	2.0
C1-O3	93.0	1.250	2.0
O3-C4 (TS)	90.0	1.677	2.0
C4-O5 (RS)	93.0	1.250	2.0
C4-O5 (TS)	90.0	1.270	2.0
C4-N6 (RS)	93.9	1.400	2.0
C4-N6 (TS)	90.0	1.470	2.0

C7-N6	93.9	1.400	2.0
C7-C8	96.0	1.540	0.8
O13-H14 (RS)	102.0	0.960	2.0
O13-H14 (TS)	90.0	1.057	2.0
N6-H14 (TS)	90.0	1.517	2.0

c- Angle Parameters; $V_\theta(\theta) = 1/2\, k_\theta(\theta - \theta_0)^2$

Angle type	½ k_θ	θ_0
C-N-C	50.0	109.5
C-C-N	50.0	109.5
N-C-O	50.0	120.0
H-C-C	50.0	109.5
H-C-H	50.0	109.5
H-C-N	50.0	109.5
O-C-O	50.0	120.0
C-O-C	80.0	109.5
O-C-C	50.0	120.0
C-O-H (RS)	80.0	109.5
C-O-H (TS)	50.0	120.0

d-. Dihedral parameters; $V_\varphi(\varphi) = k_\varphi(1 + \cos(n\varphi - \varphi_0))$

Dihedral Type	k_φ	n	φ_0
H-C-C-H	1.0	3.0	0.0
O-C-N-C	15.0	2.0	180.0
H-C-N-C	2.0	3.0	0.0
C-N-C-C	2.0	3.0	0.0
C-N-C-H	2.0	3.0	0.0
N-C-C-H	2.0	3.0	0.0
H-N-C-C	2.0	3.0	0.0
H-N-C-H	2.0	3.0	0.0
C-C-O-C	15.0	2.0	180.0
O-C-O-C	15.0	2.0	180.0
O-C-C-C	15.0	2.0	180.0

e-. Improper torsion parameters; $V_\varphi(\varphi) = k_\varphi(1 + \cos(n\varphi - \varphi_0))$

Dihedral Type	k_φ	n	φ_0
C-C-O-N	30.0	2	180.0
C-C-C-C	30.0	2	180.0
C-C-O-O	30.0	2	180.0
N-C-C-C	30.0	2	180.0
N-C-C-H	30.0	2	180.0

f- Nonbonded Parameters (EVB atom wise parameters for atoms bonded in one of the EVB states)

$$V_{nb} = \sqrt{C_A * C_B}\, e^{-\sqrt{\beta_A \beta_b}*r}$$

Atom Type	C	β
H0	5.0	2.5
C+	91.0	2.5
N+	60.0	2.5
O0	53.0	2.5
O-	90.0	2.5
C0	91.0	2.5

g- Nonbonded Parameters (EVB atom wise parameters for atoms never bonded)

$$V_{nb} = \frac{vdwa_A * vdwa_B}{r^{12}} - \frac{vdwb_A * vdwb_B}{r^6}$$

Atom Type	vdwa	vbwb
H0	7.0	0.0
C+	632.0	24.0
N+	774.0	24.0
O0	774.0	24.0
O-	1400.0	24.0
C0	632.0	24.0

Other EVB parameters

gas shift 2, α = -7.3

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