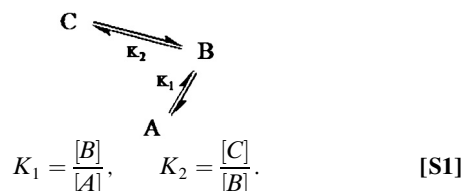


# Supporting Information

Nagel et al. 10.1073/pnas.1104989108

## SI Text

**Analyses in the Case of More than Two Conformational States. Case I:** Here, we consider a case of conformational sampling in which the enzyme must go through multiple inactive conformational states  $A$  and  $B$  to reach the active state  $C$ :



We obtain an expression for the fraction of enzyme in the active conformer ( $C$ ) as follows in Eqs. S2–S6:

$$\frac{[C]}{[A]} = K_1 K_2 = \frac{[C]}{E_{\text{TOT}} - [B] - [C]}, \quad [\text{S2}]$$

$$\frac{1}{K_1 K_2} = \frac{E_{\text{TOT}} - [B] - [C]}{[C]} = \frac{E_{\text{TOT}}}{[C]} - \frac{1}{K_2} - 1, \quad [\text{S3}]$$

$$\frac{[C]}{E_{\text{TOT}}} = \frac{1}{\frac{1}{K_1 K_2} + \frac{1}{K_2} + 1} = \frac{K_1 K_2}{1 + K_1 + K_1 K_2}, \quad [\text{S4}]$$

$$k_{\text{obs}} = \frac{K_1 K_2}{1 + K_1 + K_1 K_2} \times A_{\text{int}} e^{-E_a/RT}. \quad [\text{S5}]$$

When  $K_1$  and  $K_1 K_2 \ll 1$ , at low temperature, we obtain Eqs. S6–S8:

$$\ln(k_{\text{obs}}) = \ln(K_1 K_2) + \ln(A_{\text{int}}) - \frac{E_a(\text{int})}{RT}, \quad [\text{S6}]$$

$$\ln(k_{\text{obs}}) = \ln(A_{\text{int}}) + \frac{\Delta S_{c,1}^{\circ} + \Delta S_{c,2}^{\circ}}{R} - \frac{E_a(\text{int}) + \Delta H_{c,1}^{\circ} + \Delta H_{c,2}^{\circ}}{RT}, \quad [\text{S7}]$$

$$\ln(A_{\text{obs}}) = \ln(A_{\text{int}}) + \frac{\Delta S_{c,1}^{\circ} + \Delta S_{c,2}^{\circ}}{R}. \quad [\text{S8}]$$

**Case II:** For the case of two (or more) active conformers  $B$  and  $C$  that are in equilibrium with an inactive conformer  $A$ , we have:



With

$$K_1 = \frac{[B]}{[A]}, \quad K_2 = \frac{[C]}{[A]}. \quad [\text{S9}]$$

For the simplest case, where  $C$  and  $B$  have the same rate constant, we can solve the problem in terms of their joint concentration.

We first set up a virtual species  $[X] = [B] + [C]$ , with  $K_x = [X]/[A]$ , and rearrange as follows:

$$[X] = (E_{\text{TOT}} - [X])K_x, \quad [\text{S10}]$$

$$[X](1 + K_x) = E_{\text{TOT}}K_x, \quad [\text{S11}]$$

$$\frac{[X]}{E_{\text{TOT}}} = \frac{K_x}{1 + K_x}. \quad [\text{S12}]$$

Because  $[X] = [B] + [C]$ ,  $K_x = [B]/[A] + [C]/[A]$ , so

$$\frac{[X]}{E_{\text{TOT}}} = f_{\text{active}} = \frac{K_1 + K_2}{1 + K_1 + K_2}, \quad [\text{S13}]$$

$$k_{\text{obs}} = \frac{K_1 + K_2}{1 + K_1 + K_2} \times A_{\text{int}} e^{-E_a(\text{int})/RT}, \quad [\text{S14}]$$

and the logarithmic form is now

$$\ln(k_{\text{obs}}) = \ln(K_1 + K_2) - \ln(1 + K_1 + K_2) + \ln(A_{\text{int}}) - \frac{E_a(\text{int})}{RT}. \quad [\text{S15}]$$

At low temperature,  $K_1$  and  $K_2$  are small, and the term  $\ln(1 + K_1 + K_2)$  drops out. Although the form of  $\ln(K_1 + K_2)$  is not obvious, we can consider limiting cases. In one case, we may have  $K_1$  and  $K_2$  that are essentially identical, so that the first term becomes  $\ln(2K_1) = \ln(2) + \ln(K_1)$ . Similar to the case for a single conformer, now

$$\ln(k_{\text{obs}}) = \ln(2) + \ln(A_{\text{int}}) + \frac{\Delta S_{c,1}^{\circ}}{R} - \frac{E_a(\text{int}) + \Delta H_{c,1}^{\circ}}{RT}. \quad [\text{S16}]$$

In this way, it can be seen that for multiple active conformers, there is an additional contribution to the Arrhenius prefactor:

$$\ln(A_{\text{obs}}) = \ln(2) + \ln(A_{\text{int}}) + \frac{\Delta S_{c,1}^{\circ}}{R}. \quad [\text{S17}]$$

And generally, for  $n$  identical active conformers, we have

$$\ln(A_{\text{obs}}) = \ln(n) + \ln(A_{\text{int}}) + \frac{\Delta S_c^{\circ}}{R}. \quad [\text{S18}]$$

Likewise, in the opposite extreme, if one active conformer is much more energetically favorable than the other (e.g.,  $K_1 \gg K_2$ ), then the lower energy conformer dominates, and the problem simplifies to *Case I* above.

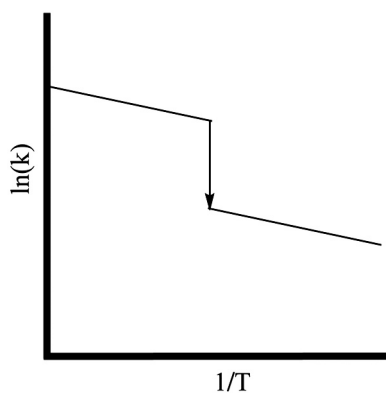


Fig. S1. An Arrhenius plot representing a hypothetical conformational change that leads to an irreversible loss of the active form of enzyme below the temperature break.

Table S1. Kinetic parameters for ht-ADH variants

Temperature, °C	$k_{cat}$ , $s^{-1}$	Error	Temperature, °C	$k_{cat}$ , $s^{-1}$	Error
L176V			L176Δ		
12.5	0.12	0.01	10	0.007	0.001
16	0.25	0.02	15	0.017	0.004
20	0.48	0.03	20	0.065	0.026
24	0.89	0.08	30	0.43	0.10
28.5	1.7	0.1	40	0.91	0.17
33	2.4	0.2	50	1.8	0.3
37.5	4.1	0.3	60	3.1	1.0
42	6.0	0.6			
45	8.9	0.7			
52	14.6	1.3			
L176A			V260A		
5	0.07	0.01	10	0.044	0.004
12.5	0.26	0.05	15	0.128	0.010
20	0.81	0.07	20	0.41	0.02
28.5	2.9	0.3	30	2.4	0.2
33	4.2	0.4	37.5	7.5	0.8
37.5	6.6	0.7	45	14.2	0.7
45	11.9	1.7	50	18.1	2.2
52	19.1	2.5	58	32.8	3.0
L176G					
10	0.32	0.04			
16	0.97	0.12			
20	1.8	0.3			
30	7.7	1.4			
37.5	15.2	3.6			
46	29.4	3.5			
52	51.0	8.2			