The uncatalyzed rate of peptide bond hydrolysis is $k_{non} = 1.5 \times 10^{-9}\ s^{-1}$. Addition of an enzyme can give a rate of $k_{enzyme} = 120\ s^{-1}$. How much did the enzyme lower the transition state energy barrier?

Reaction rate in terms of barrier height:

In the absence of enzyme, the reaction is:

$$S \rightleftharpoons TS \rightarrow P$$

The rate-limiting step is accessing the transition state, so we're only going to think about the $S \rightleftharpoons TS$ part of the reaction. The rate of this reaction is given by:

$$k_{S \rightarrow TS} = k_{try} P_{TS}. \tag{1}$$

This is just like rolling a dice. The rate of seeing sixes depends on how often you roll (k_{try}) times the probability of getting a six on a given roll (P_{TS}). We're going to ignore k_{try} because it is going to disappear later. The probability we're in TS (P_{TS}) is just the fraction of the population in TS rather than S[1]:

$$P_{TS} = \frac{[TS]}{[S] + [TS]}.$$

If the transition state is much less stable than the starting state (as it usually is), $[S] \gg [TS]$. This means that:

$$P_{TS} \approx \frac{[TS]}{[S]}.$$

This looks a lot like an equilibrium constant for the $S \rightleftharpoons TS$ reaction. If we treat it as such, we can describe this in terms of free energy:

$$P_{TS} \approx \frac{[TS]}{[S]} = K_{S \rightarrow TS}$$

$$\Delta G_{S \rightarrow TS} = -RTln(K_{S \rightarrow TS}).$$

Now, rearrange:

$$\frac{-\Delta G_{S \rightarrow TS}}{RT} = ln(K_{S \rightarrow TS})$$

$$e^{-\Delta G_{S \rightarrow TS}/RT} = K_{S \rightarrow TS} \approx P_{TS}.$$

Finally, substitute this back into equation 1 to obtain:

$$k_{S \rightarrow TS} = k_{try} e^{-\Delta G_{S \rightarrow TS}/RT}. \tag{2}$$

This says that the rate of a reaction depends *exponentially* on the free energy difference between the starting and transition states.

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[1] We're actually being rather tricky here and making a couple of assumptions. First, we're saying the $S \rightleftharpoons TS$ reaction is at "equilibrium" (not always a great assumption). Second, we're assuming that there is no "momentum" in the reaction. A molecule that hops up to TS could just as likely fall back to S than P. This quickly gets into deep weeds, but the basic exponential relationship we'll derive holds even when we relax these assumptions.

Comparing rates

Okay, we now want to understand how much the enzyme stabilized the transition state to give the observed rate increase. This is basically:

$$\Delta\Delta G = \Delta G_{S\to TS,enzyme} - \Delta G_{S\to TS,non}.$$

Let's start by thinking about the ratio of rates. The speed up is given by:

$$\frac{k_{enzyme}}{k_{non}}.$$

We can substitute our result from equation 2 in to obtain:

$$\frac{k_{enzyme}}{k_{non}} = \frac{k_{try}e^{-\Delta G_{S\to TS,enzyme}/RT}}{k_{try}e^{-\Delta G_{S\to TS,non}/RT}}.$$

k_{try} cancels:

$$\frac{k_{enzyme}}{k_{non}} = \frac{e^{-\Delta G_{S\to TS,enzyme}/RT}}{e^{-\Delta G_{S\to TS,non}/RT}}.$$

Using the log rule that $x^{-y} = 1/x^y$ we can write:

$$\frac{k_{enzyme}}{k_{non}} = e^{-\Delta G_{S\to TS,enzyme}/RT}e^{\Delta G_{S\to TS,non}/RT}.$$

Using the log rule that $x^a x^b = x^{a+b}$ we can write:

$$\frac{k_{enzyme}}{k_{non}} = e^{-\Delta G_{S\to TS,enzyme}/RT+\Delta G_{S\to TS,non}/RT}.$$

Now, factor and rearrange:

$$\frac{k_{enzyme}}{k_{non}} = e^{(\Delta G_{S\to TS,non}-\Delta G_{S\to TS,enzyme})/RT}.$$

We're interested in

$$\Delta\Delta G = \Delta G_{S\to TS,enzyme} - \Delta G_{S\to TS,non},$$

so substitute this in:

$$\frac{k_{enzyme}}{k_{non}} = e^{-\Delta\Delta G/RT}.$$

This is a cool result. It says that the rate speed up of an enzyme depends *exponentially* on the amount is lowers the transition state energy. Now, solve for $\Delta\Delta G$:

$$ln\left(\frac{k_{enzyme}}{k_{non}}\right) = -\Delta\Delta G/RT$$

$$-RTln\left(\frac{k_{enzyme}}{k_{non}}\right) = -\Delta\Delta G,$$

and put in numbers:

$$-0.0083 \; kJ \cdot mol^{-1} \cdot K^{-1} \times 300 \; K \times ln \left(\frac{120 \; s^{-1}}{1.5 \times 10^{-9} \; s^{-1}} \right) = -62.5 \; kJ \cdot mol^{-1}.$$

This tells us that lowering the transition state energy by $-62.5 \; kJ{\cdot}mol^{-1}$—about 3 hydrogen bonds—gives a 100-billion fold speed up in rate.

Small **perturbations to the transition state can lead to** *large* **changes in rate.**