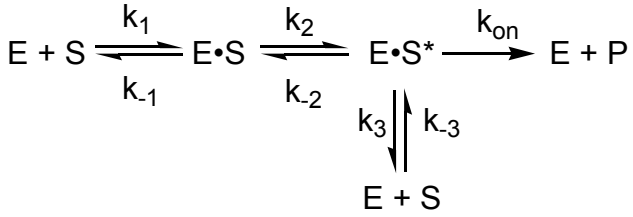
The Hopfield Mechanism

Consider standard Michaelis-Menten Kinetics, the greatest discrimination (f) that one can have between cognate (C) and non-cognate (N) substrates is:

$$f = \frac{\left(k_{cat}/K_m\right)^C}{\left(k_{cat}/K_m\right)^N}$$

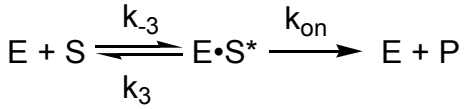
which we have seen can produce about a 250 fold preference for isoleucine over valine in IleRS, but which does not consistently give the > 10,000 fold preference required for high-fidelity enzymatic processes with multiple possible substrates.

Hopfield noted that a second discrimination step in an enzymatic mechanism is possible:



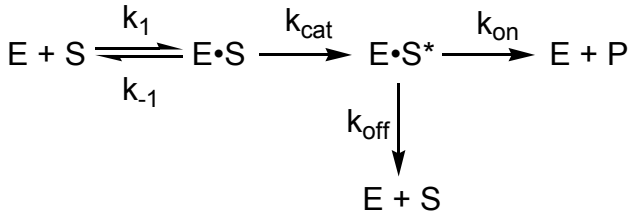
In this instance the E•S complex is placed in equilibrium with a new discrimination site to create E•S* before final catalysis. E•S* can either dissociate with a new equilibrium constant or go on to produce product.

The problem with the mechanism is that it really only substitutes a new single intermediate path for the reaction mechanism, which by-passes the original k_1/k_{-1} binding step:



So again, we get another situation in which discrimination is defined by the second order overall rate constant for this process ($k_{on}/(k_3 + k_{on})/k_{-3}$).

Hopfield noted, however, that the second discrimination step could work if we make the dissociation of E•S* irreversible:



Note that I have also make the k_2 step (now k_{cat}) irreversible. That will help the kinetic derivation along later, and it also reflects how things happen in these systems. Let's see how this helps our story. Our preliminary rate law will be:

rate = $k_{on}[E \bullet S^*]$

but we can't leave an intermediate in the rate law, so let's use the steady state assumption:

$$\frac{d[E \bullet S^*]}{dt} = 0 = \frac{k_{cat}}{K_m}[E][S] - k_{off}[E \bullet S^*] - k_{on}[E \bullet S^*]$$

$$[E \bullet S^*] = \frac{\left(k_{cat} / K_m\right)[E][S]}{(k_{on} + k_{off})}$$

That gives the rate law:

$$\text{rate} = \frac{k_{on}\left(k_{cat} / K_m\right)}{(k_{on} + k_{off})}[E][S]$$

Now, consider the cognate and non-cognate substrates, C and N. Each of them will have different rate constants, but importantly, for the cognate, $k_{on} >> k_{off}$, so:

$$\text{rate}^C = \left(k_{cat} / K_m\right)^C [E][C]$$

But for the non-cognate, $k_{off} >> k_{on}$ if all is working properly, so:

$$\text{rate}^N = \left(k_{cat} / K_m\right)^N \left(\frac{k_{on}^N}{k_{off}^N}\right)[E][N]$$

Thus, the discrimination now takes into account the discrimination against N in the second site (assuming [C] = [N]):

$$f = \frac{\left(k_{cat} / K_m\right)^C}{\left(k_{cat} / K_m\right)^N} \cdot \frac{k_{off}^N}{k_{on}^N}$$

Now we have two discrimination steps – the original k_{cat}/K_m difference and the difference in editing vs. forward rate for the non-cognate.

That's the kinetic mechanism, now let's look at the means of achieving it. Most important is the irreversibility of the release from E•S*. That means E•S* must be a

higher energy state that favors dissociation far over association. How does one achieve that? Through hydrolysis of ATP in aaRS's or GTP in the ribosome.