Complications to Enzyme Kinetics

- Many models, with different parameters, can result in the same functional form as Michaelis-Menten

- Can only detect slowest step

- Forward and reverse reactions can help, but intermediates may complicate interpretation

Example: Model Degeneracy

- Consider this scheme:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \overset{k_2}{\rightarrow} EP \overset{k_3}{\rightarrow} P + E$$

- Solution (when ES, EP at steady-state):

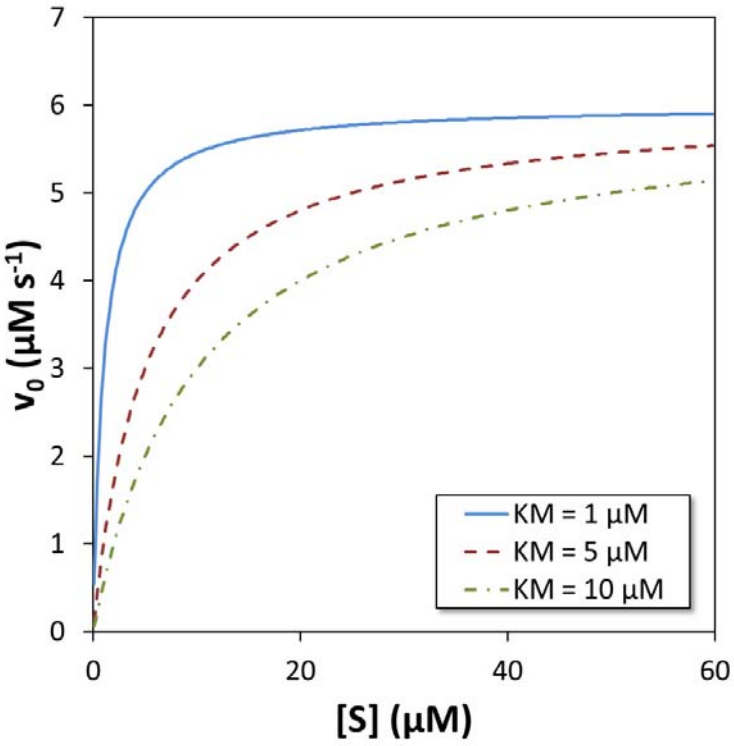
$$v = \frac{v'_{max}[S_0]}{[S_0]+K'_M} \text{ where } v'_{max} = \frac{k_2 k_3 [E_0]}{k_2+k_3} \text{ and } K'_M = \frac{k_3(k_{-1}+k_2)}{k_1(k_2+k_3)}$$

- You can't tell the difference at steady state!
  - Relaxation methods, non-reactive transition state analogs can help you pin down rates

What does K_M mean?

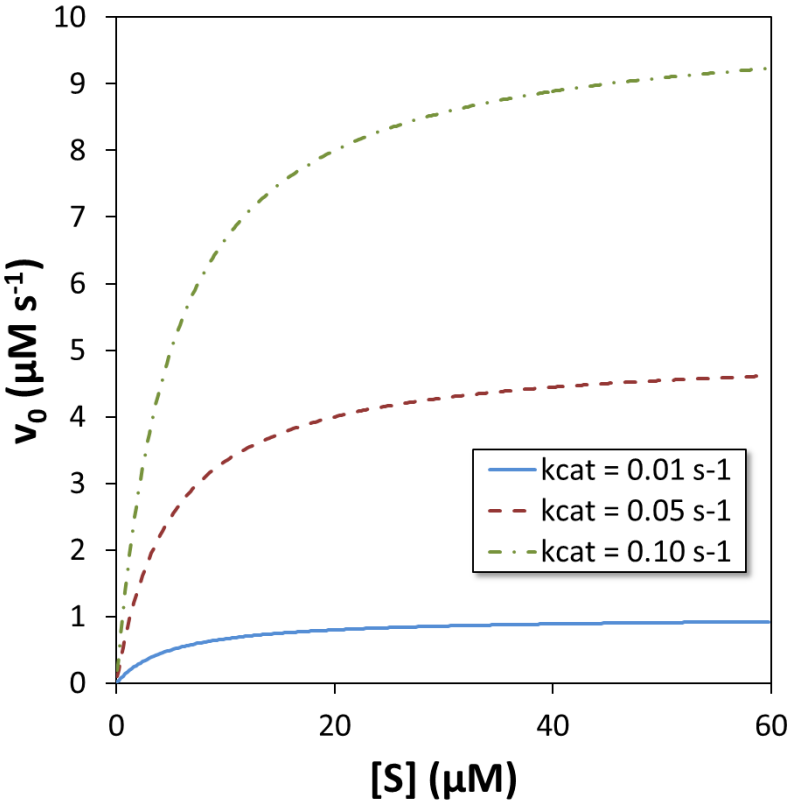
- How "tightly" does E bind to S?
- Lower K_M values will reach v_{max} sooner
- Often tuned to physiologically-relevant concentration
- When $k_{cat} \ll k_{-1}$ then

$$K_M = \frac{k_{cat} + k_{-1}}{k_1} \approx \frac{k_{-1}}{k_1} = K_d$$



What does k_{cat} mean?

- How efficient is the enzyme once a complex is formed?
- Higher k_{cat} will have a faster velocity



Enzyme Specificity

- One enzyme, two substrates: the "free" enzyme matters ($[E]$ vs. $[E_0]$)

$$v_0 = \left(\frac{k_{cat}}{K_M}\right)[E][S]$$

- Ratio of velocities determines which substrate "wins"

$$\frac{v_A}{v_B} = \frac{(k_{cat}/K_M)_A[A]}{(k_{cat}/K_M)_B[B]}$$

- If $[A]$ and $[B]$ are equal, k_{cat}/K_M is all that matters

k_{cat}/K_M: The Specificity Constant

Table 14-1 Values of K_M, k_{cat}, and k_{cat}/K_M for Some Enzymes and Substrates

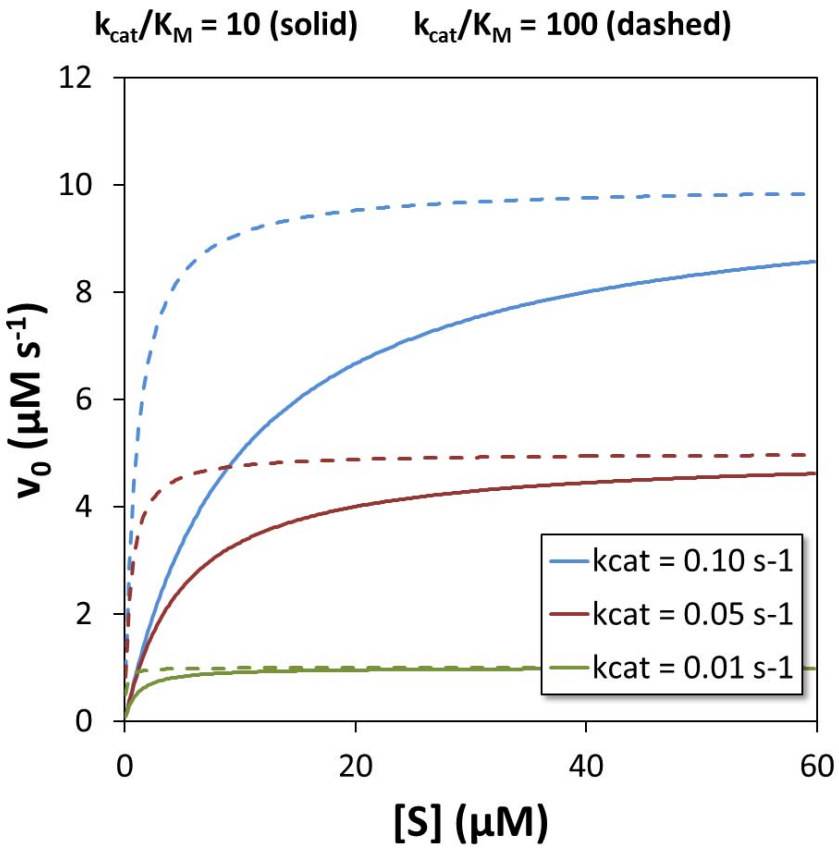
Enzyme	Substrate	K_M (M)	k_{cat} (s^{-1})	k_{cat}/K_M ($M^{-1} \cdot s^{-1}$)
Acetylcholinesterase	Acetylcholine	9.5×10^{-5}	1.4×10^{4}	1.5×10^{8}
Carbonic anhydrase	CO_2	1.2×10^{-2}	1.0×10^{6}	8.3×10^{7}
	HCO_3^-	2.6×10^{-2}	4.0×10^{5}	1.5×10^{7}
Catalase	H_2O_2	2.5×10^{-2}	1.0×10^{7}	4.0×10^{8}
Chymotrypsin	*N*-Acetylglycine ethyl ester	4.4×10^{-1}	5.1×10^{-2}	1.2×10^{-1}
	N-Acetylvaline ethyl ester	8.8×10^{-2}	1.7×10^{-1}	1.9
	N-Acetyltyrosine ethyl ester	6.6×10^{-4}	1.9×10^{2}	2.9×10^{5}
Fumarase	Fumarate	5.0×10^{-6}	8.0×10^{2}	1.6×10^{8}
	Malate	2.5×10^{-5}	9.0×10^{2}	3.6×10^{7}
Superoxide dismutase	Superoxide ion ($O_2^{\cdot -}$)	3.6×10^{-4}	1.0×10^{6}	2.8×10^{9}
Urease	Urea	2.5×10^{-2}	1.0×10^{4}	4.0×10^{5}

- Higher values are more “specific”
- Diffusion limited $k_{cat}/K_M \approx 10^7 - 10^8\ \mathrm{M^{-1}\ s^{-1}}$

Voet and Voet. *Biochemistry, 4th ed.* p. 489.

What does specificity mean?

- Enzyme may be fast or slow
- Determines the "squareness" of the curve
- More specific enzymes will steeply reach v_{max} without "trailing-off"



Enzyme Inhibition

- **Substrate competition:** One substrate can overwhelm another based on k_{cat}/K_M
- **Competitive inhibition:** Inhibitor binds to enzyme, blocking access to substrate
- **Noncompetitive inhibition:** Inhibitor binds to enzyme regardless of whether substrate is bound
- **Allosteric regulation:** Enzyme can be activated or inhibited by modulating substrate binding

Competitive Inhibition

- Scheme:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_{cat}} P + E$$

$$EI \overset{K_I}{\rightleftarrows} E + I$$

- Result:

$$v = \frac{v_{max}\,[S_0]}{[S_0] + K'_M} \text{ where } K'_M = K_M\left(1 + \frac{[I]}{K_I}\right)$$

Competitive Inhibition: Derivation

- We need an expression for [ES], since:

$$\frac{d[P]}{dt} = k_{cat}[ES]$$

- We have an expression for [ES] at steady state:

$$\frac{d[ES]}{dt} = 0 = k_1[E][S] - (k_{-1} + k_{cat})[ES]$$

- But $[E]$ can't exist in our final solution, only $[E_0]$. Use conservation of mass:

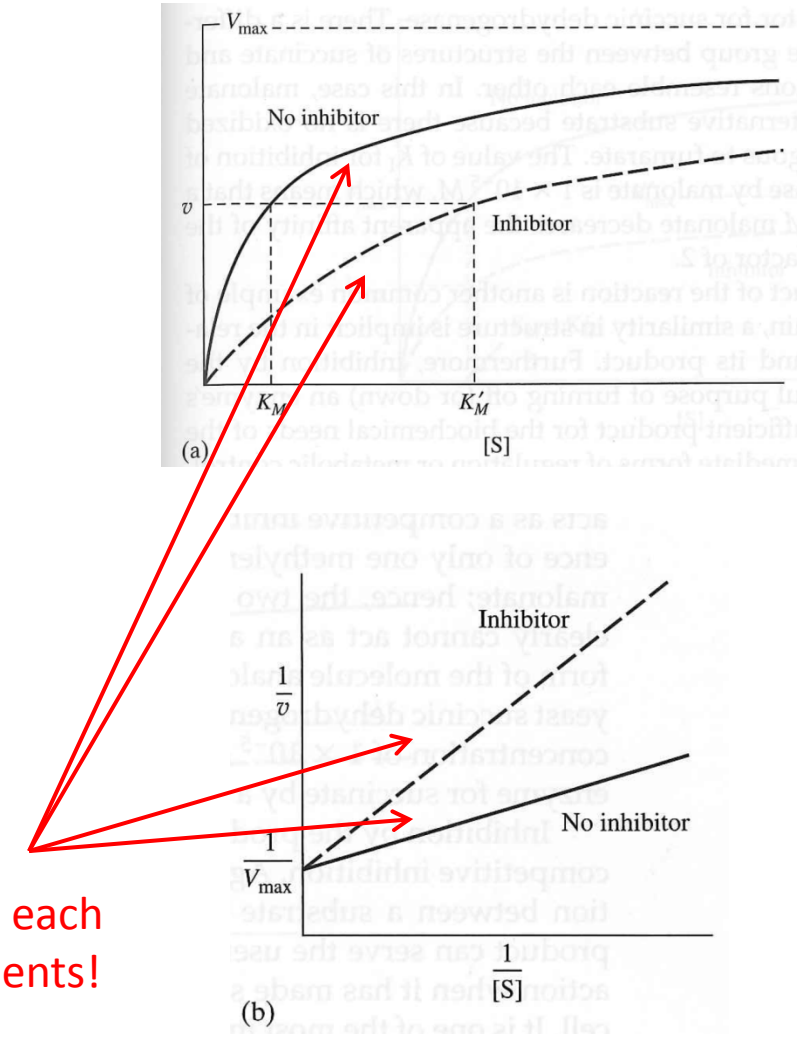
$$[E_0] = [E] + [ES] + [EI]$$

- Use K_I to eliminate $[EI]$ from above expression; final expression for $[ES]$ will only contain $[E_0]$, $[S]$, and constants

Competitive Inhibition

- With competitive inhibitor, the K_M (not the v_{max}!) can be adjusted

- Adding enough $[S]$ will always overcome inhibitor



Remember: These curves are each created from *several* experiments!

Tinoco, p. 392.

Non-competitive Inhibition

- Scheme:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_{cat}} P + E$$

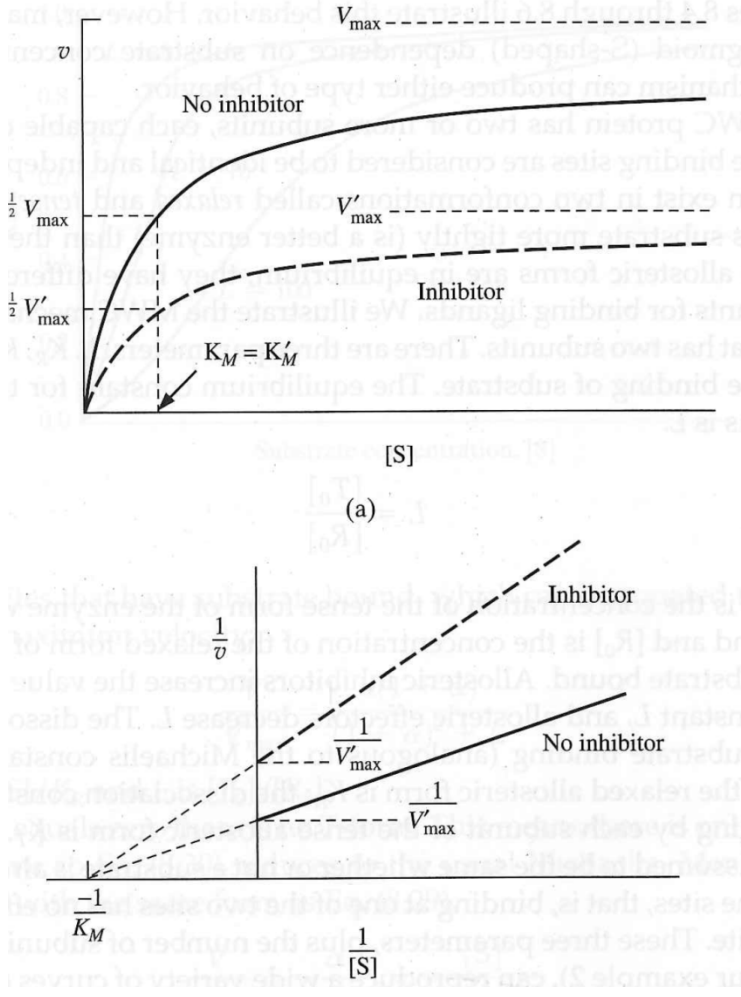
$$EI \overset{K_I}{\rightleftarrows} E + I \text{ and } ESI \overset{K_I}{\rightleftarrows} ES + I$$

- Binding of inhibitor and substrate are independent
  - But ESI cannot form product

Non-competitive Inhibition

- With non-competitive inhibitor, the v_{max} is affected, but the enzyme can still bind with the same K_M

- Adding more $[S]$ will not overcome inhibitor (no way to recover original v_{max})



Tinoco, p. 393.

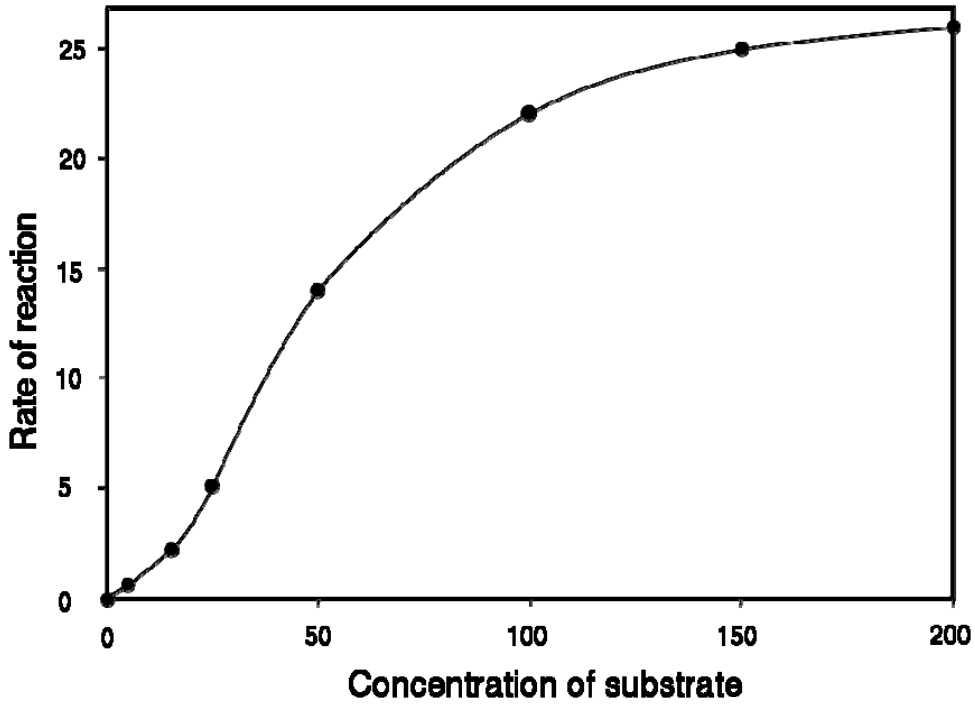
Allostery and Enzymes

- **Allostery:** Binding at one site affects the binding at another site
  - It can become more or less favorable

- Allosteric effectors always bind at another site, but they can be competitive or non-competitive

- Allostery involves *conformational change*

Allostery Example



- Non-Michaelis-Menten behavior of the rate curve
  - This should remind you of cooperativity!

"Enzyme Kinetics." *Wikipedia*.

Enzyme Kinetics and Binding

- If k_{cat} is slow compared to k_{-1}, then K_M is a true dissociation constant, and

$$\frac{v_0}{v_{max}} = \frac{[S]}{K_M + [S]}$$

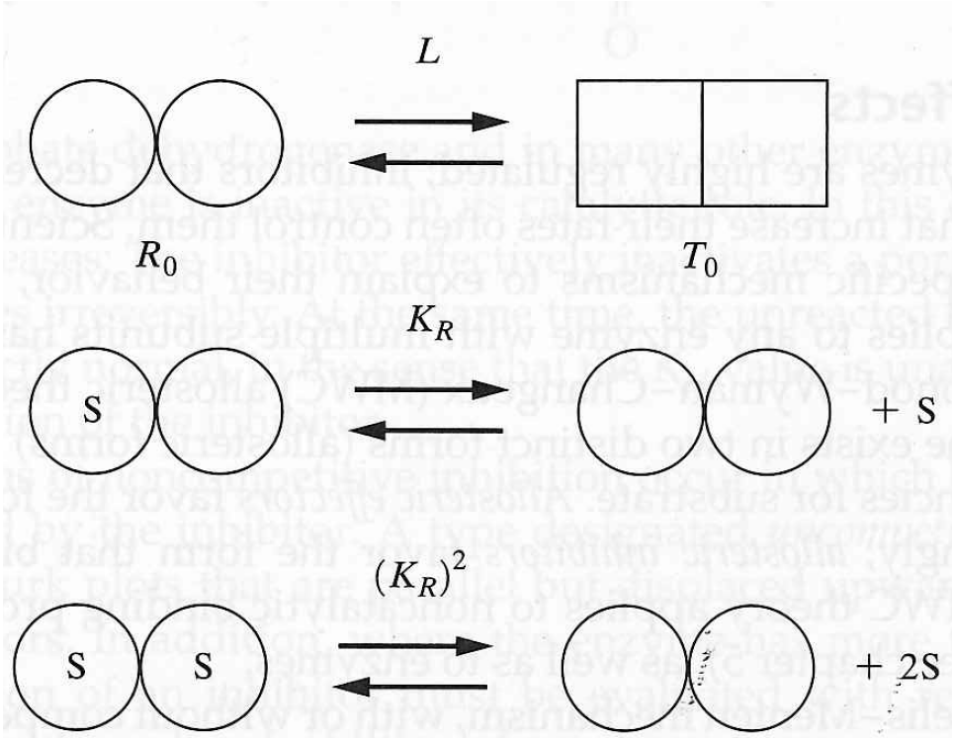
This should look eerily familiar!

- If binding is cooperative, we could expect to see more complicated expressions

Monod-Wyman-Changeux

Relaxed: binds substrate easily →

← **Tense:** does not bind substrate



Both sites bind independently →

- **MWC Model:** Alternative to our simple model for allostery (which used τ)

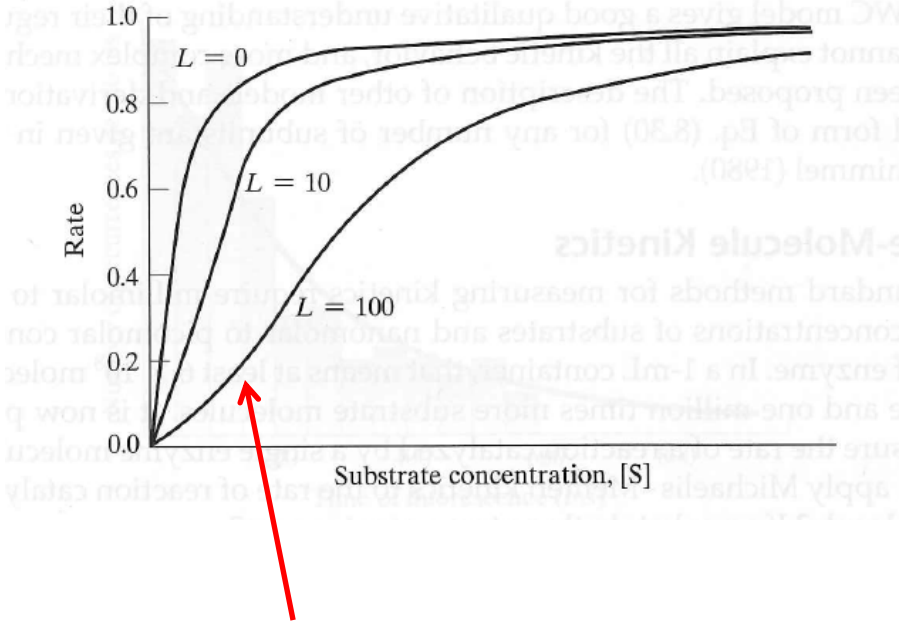
Monod-Wyman-Changeux

- Result:

$$\frac{v_0}{v_{max}} = \frac{\alpha(1+\alpha)}{(1+\alpha)^2+L}$$

- L is equilibrium between T and R ($[T_0]/[R_0]$)

- α defined as $\alpha = [S]/K_R$
  - similar to $S = K[L]$ in our previous discussion of binding



Cooperativity comes from equilibrium between R and T; substrate can shift that equilibrium

Tinoco, p. 396.