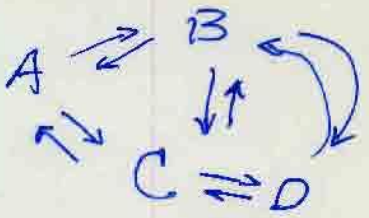
07: 34-2

MICROSCOPIC REVERSIBILITY

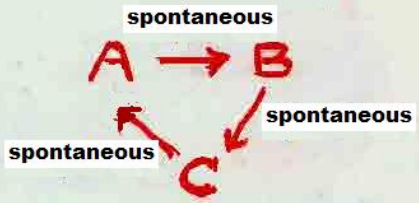
MUST ACCOUNT FOR REVERSE OF ALL PROPOSED PROCESSES



AT EQUILIBRIUM: FORWARD RATE = BACKWARD RATE

FOR ANY PAIR

NEVER



Perpetual Motion!

implies that $G_A > G_B > G_C > G_A$

A
B
C

But $\Delta G = 0$ for cyclic process.

Consider: $C \underset{K'}{\overset{K}{\rightleftharpoons}} A$

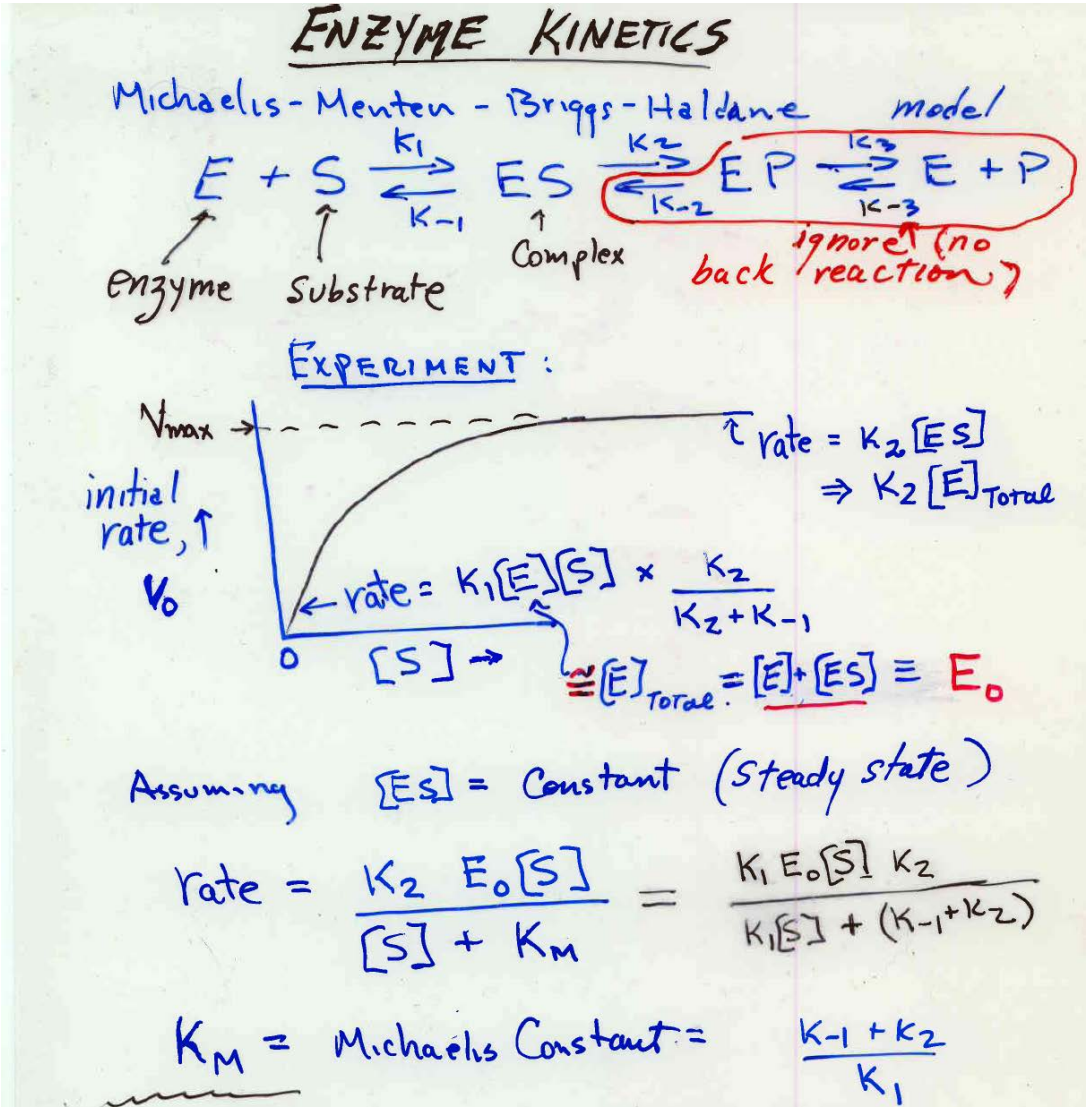
IF BOTH ARE 1st order:

Forward rate = $K[C]$

Back rate = $K'[A]$

AT EQUIL: $K[C] = K'[A]$

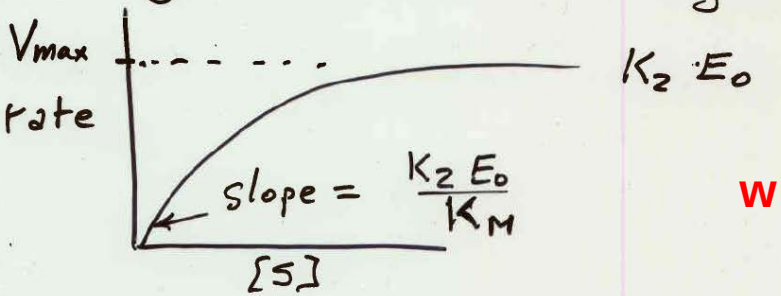
$K_{eq} = \left(\frac{[A]}{[C]}\right)_{eq} = \frac{K}{K'}$



What are the units of K_M? Concentration = mol/L

LINEWEAVER – BURKE :

Plotting rate vs. [S] gives:



when [S] very LARGE

when [S] very small

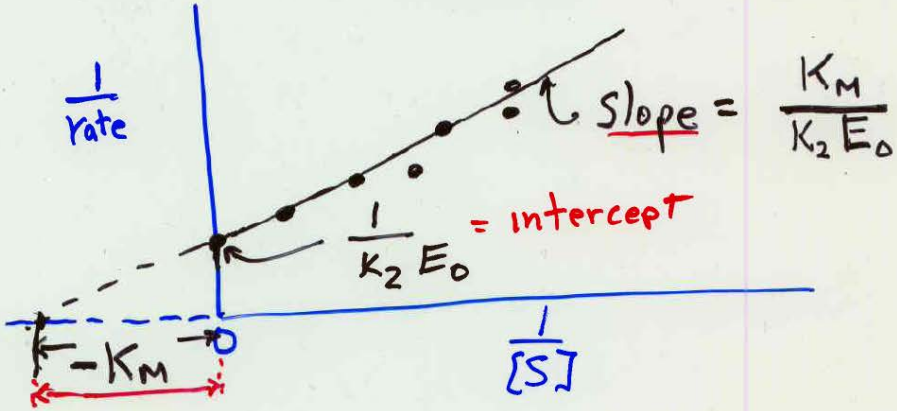
HARD TO EXTRACT K_M & K_2

$$\text{rate (initial)} = \frac{K_2 E_0 [S]}{[S] + K_M}$$

[S] cancels if very LARGE

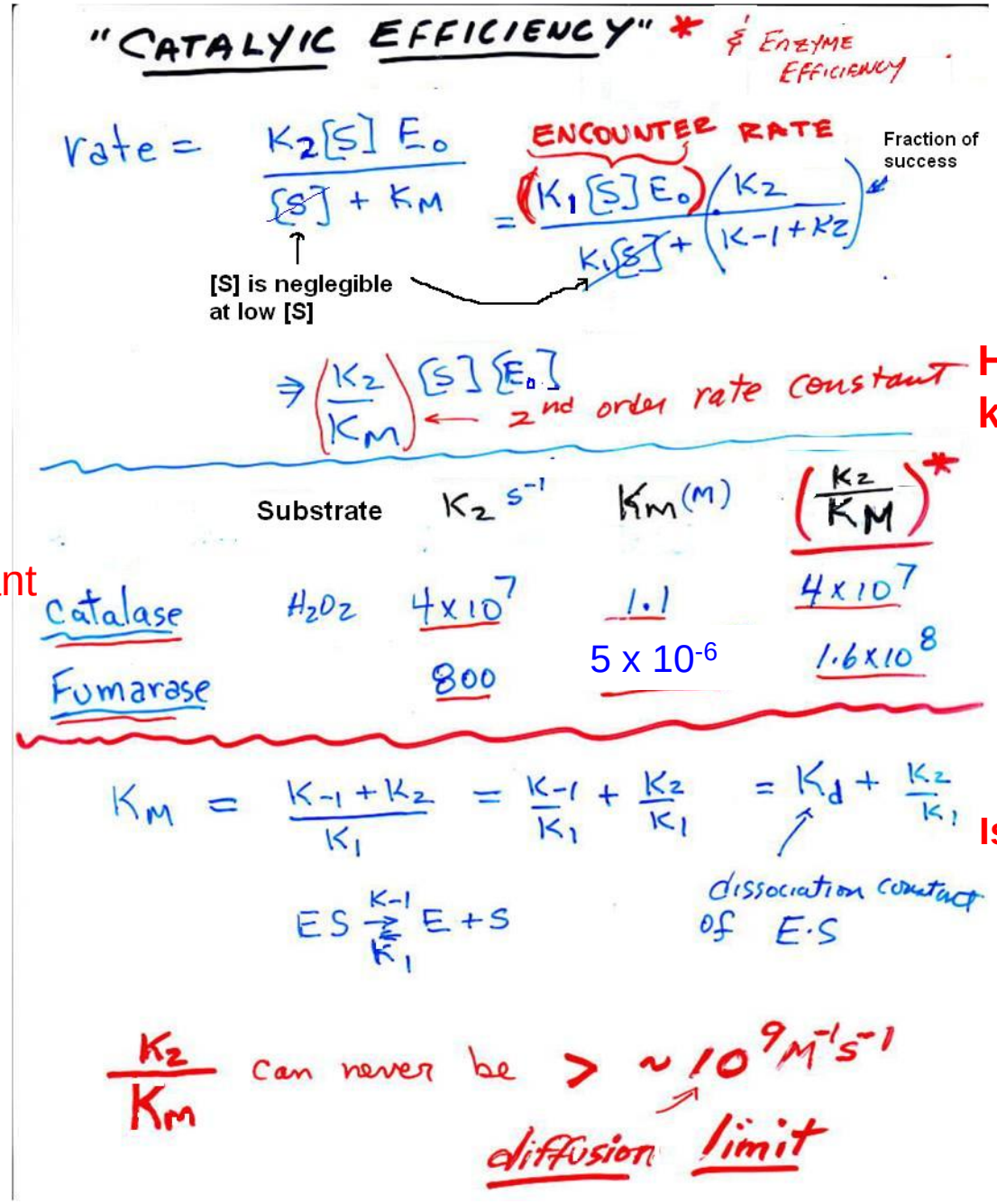
$$\frac{1}{\text{rate}} = \frac{[S] + K_M}{K_2 E_0 [S]} = \frac{1}{K_2 E_0} + \frac{K_M}{K_2 E_0 [S]}$$

equation of straight line



Limit of VERY low [S]

A small K_M is like a small dissociation constant



$$K_M = \frac{k_{-1}+k_2}{k_1}$$

How large can k_2/K_M be?

*Specificity Constant in our text

Is k_2 ever >>k_1???

Single Molecule Kinetics

This shows that a single enzyme shows an efficiency that varies in time, yet the average is the same as for a typical macroscopic solution.

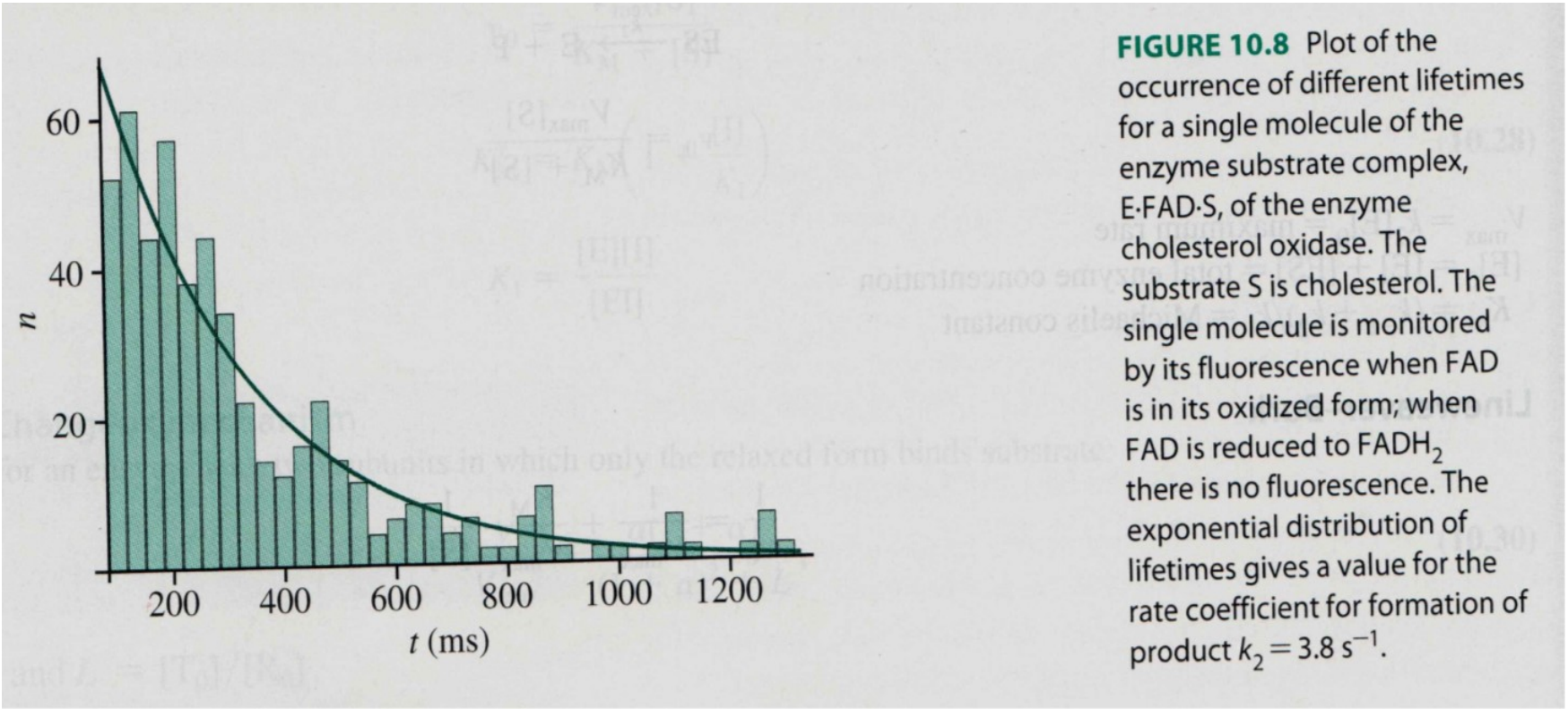


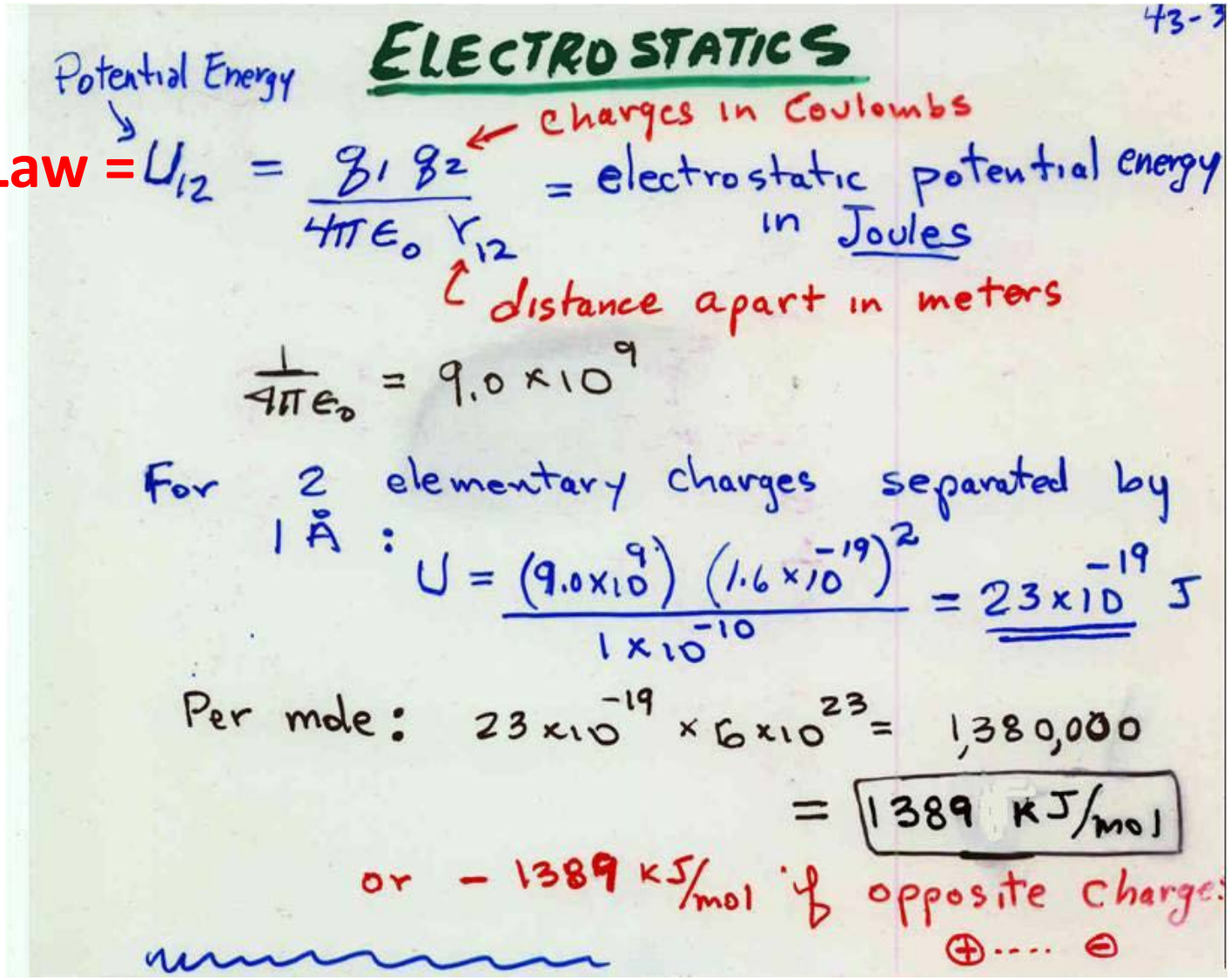
FIGURE 10.8 Plot of the occurrence of different lifetimes for a single molecule of the enzyme substrate complex, E·FAD·S, of the enzyme cholesterol oxidase. The substrate S is cholesterol. The single molecule is monitored by its fluorescence when FAD is in its oxidized form; when FAD is reduced to $FADH_2$ there is no fluorescence. The exponential distribution of lifetimes gives a value for the rate coefficient for formation of product $k_2 = 3.8\ s^{-1}$.

Arieh Warshel, stated the truth about enzyme action in 1976:

"The energy provided by protein folding reduces the activation energy by preorganizing the active site to provide large *electrostatic* stabilization of the transition state(s)."
(paraphrased)

He has preached this since 1976 and has been awarded the Nobel prize along with his mentors for associated development of modeling methods, and yet this idea still is ***not well accepted***.

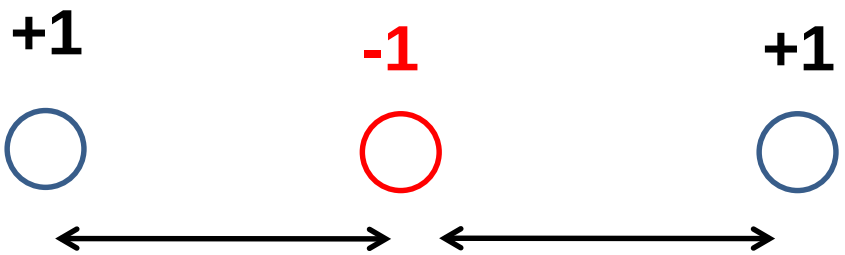
Coulomb's Law =



YOUR Coulomb's Law = 1389 kj/mol times the sum over all pairs i,j where z_i = charge in units of *e*, and r_{ij} is in Å

$$\frac{(z_i)(z_j)}{r_{ij}}$$

What about a collection of point charges?



Are charges 1 and 3 "shielded" by 2????

There really is no such thing as shielding in Coulomb's Law

$$\text{Potential Energy} = 1389\ \text{kJ/mol}\left[\frac{(z_1)(z_2)}{r_{12}} + \frac{(z_1)(z_3)}{r_{13}} + \frac{(z_2)(z_3)}{r_{23}}\right]$$

$$\text{Potential Energy} = 1389\ \text{kJ/mol}\left[\frac{-1\times 1}{1} + \frac{1\times 1}{2} \quad \frac{-1\times 1}{1}\right]$$

$$= 1389\ \text{kJ/mol}\left[-1 + 0.5 - 1\right] = 1389\ \text{kJ/mol}\left[-1.5\right] = -2084\ \text{kJ/mol}$$

This is the **only potential energy** that is in Quantum Mechanics: the Schrodinger Equation for atoms and molecules! (i.e., **CHEMISTRY**)

This is therefore what determines all **molecular** energies when determined by **quantum mechanics**.

The sum is ***ALWAYS negative*** for a ***stable*** atom or molecule.

How much is an equilibrium constant or a rate constant changed by a seemingly small electrostatic interaction ??

$$\text{Equilibrium constant}\quad K = 10^{\frac{-\Delta G^0}{2.3RT}} = 10^{\frac{-\Delta G^0}{5.7kJ}}$$

$$\text{Rate constant}\quad k = A\, e^{\frac{-E_a}{RT}} = A10^{\frac{-E_a}{5.7kJ}}$$

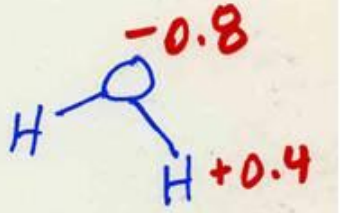
Consider a +1 and a -1 charge 100 Angstroms apart

U = 1389 x (1)(-1) / 100 = -13.9 kJ/mol

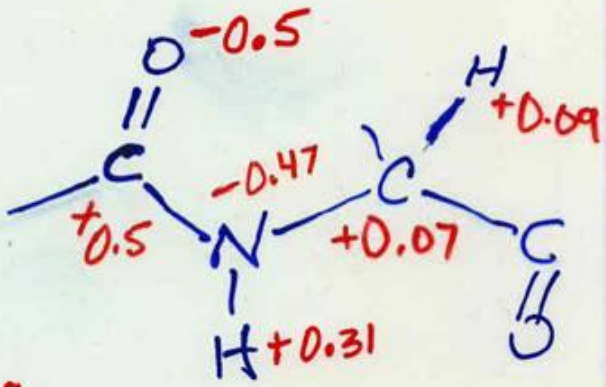
$$10^{\frac{13.9}{5.7}} = 10^{2.4} = 250 \text{ i.e., 250 times larger K!}$$

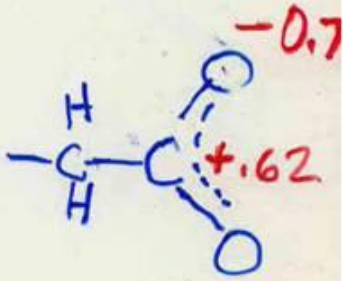
Atoms are treated as point charges and point masses.

Some typical values:

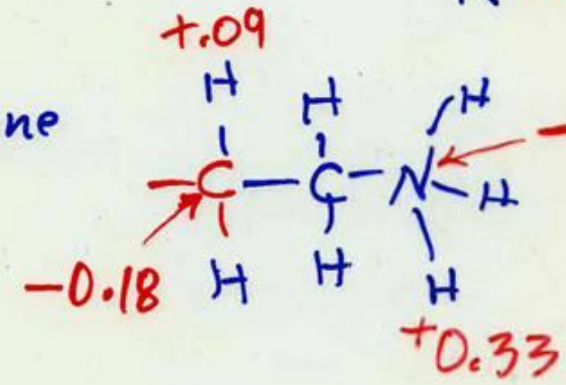


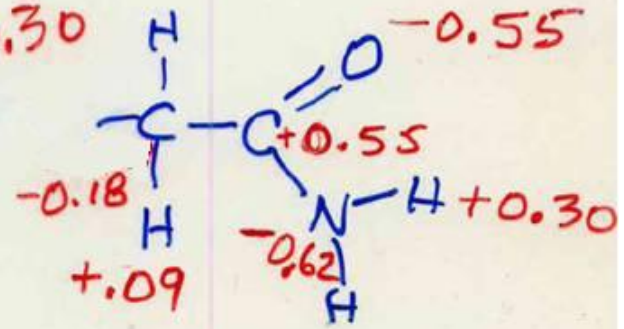
Backbone



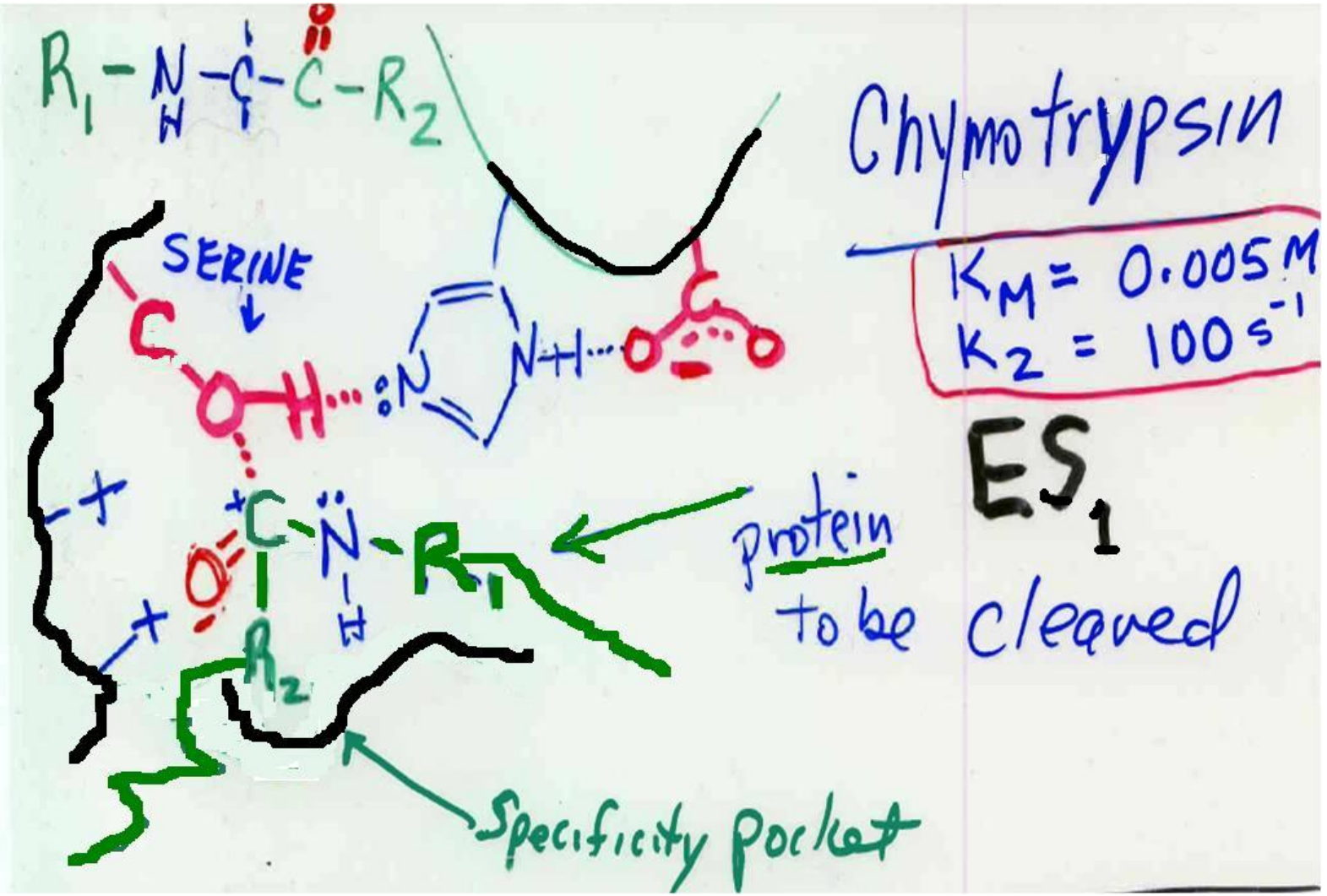


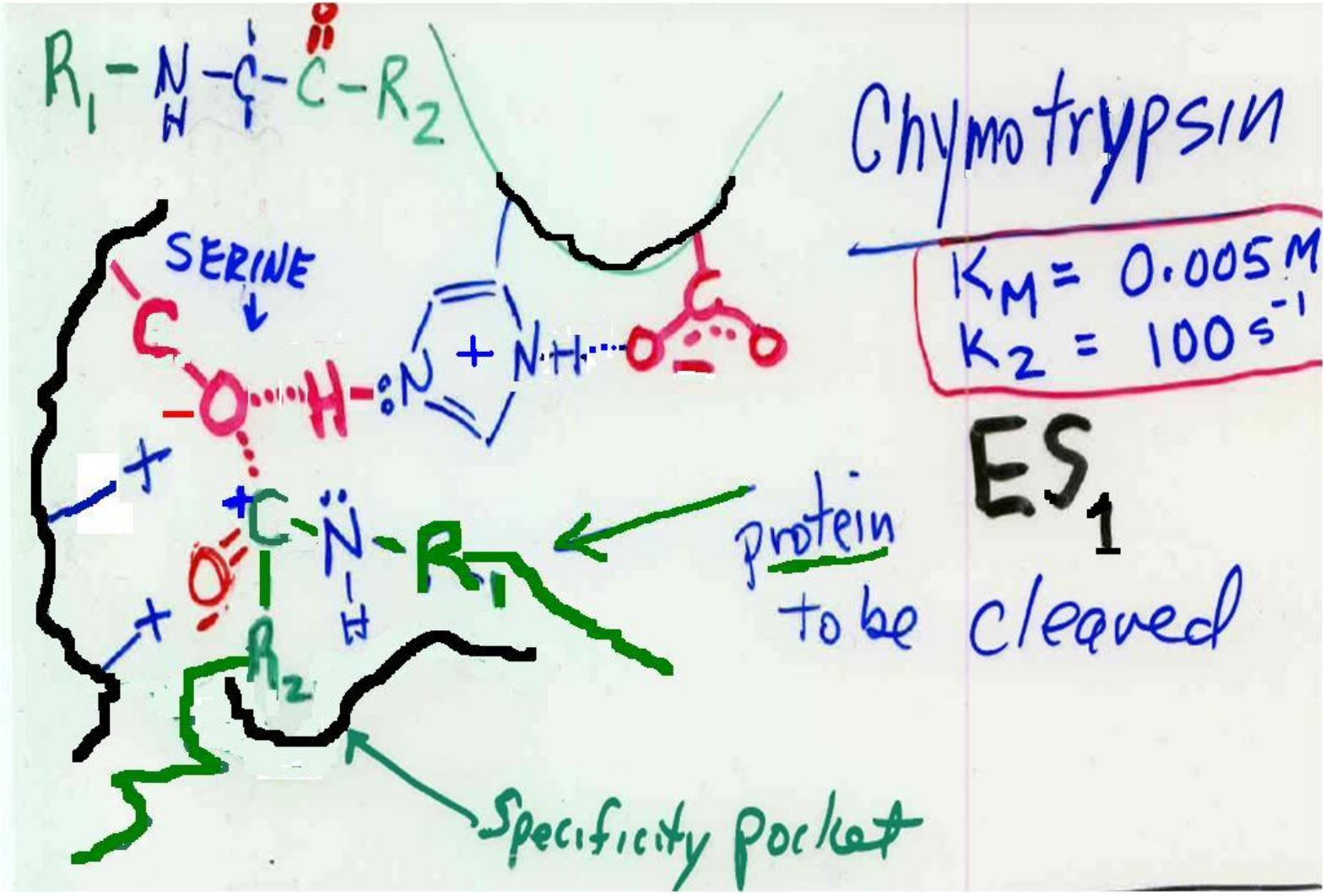
amine

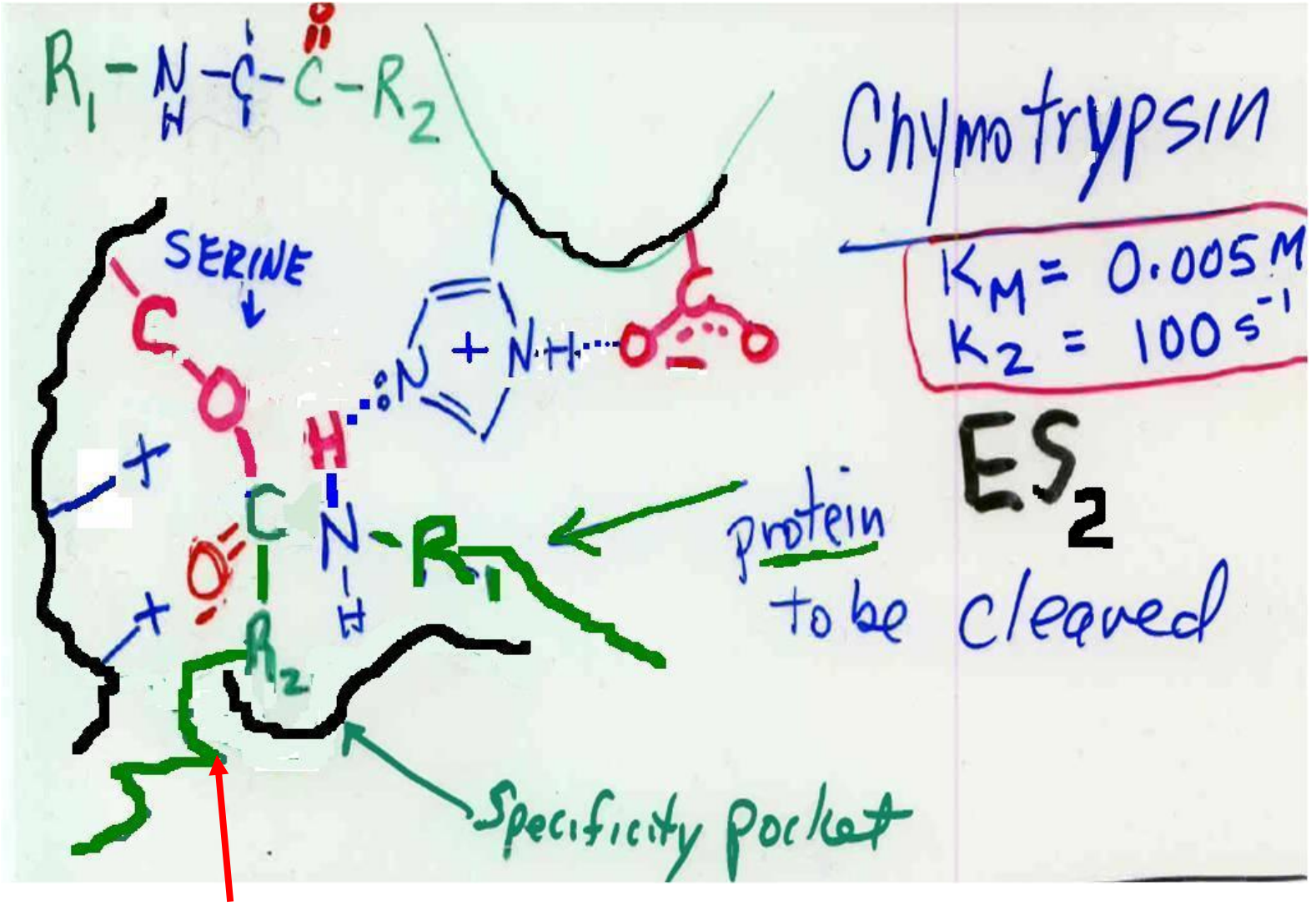




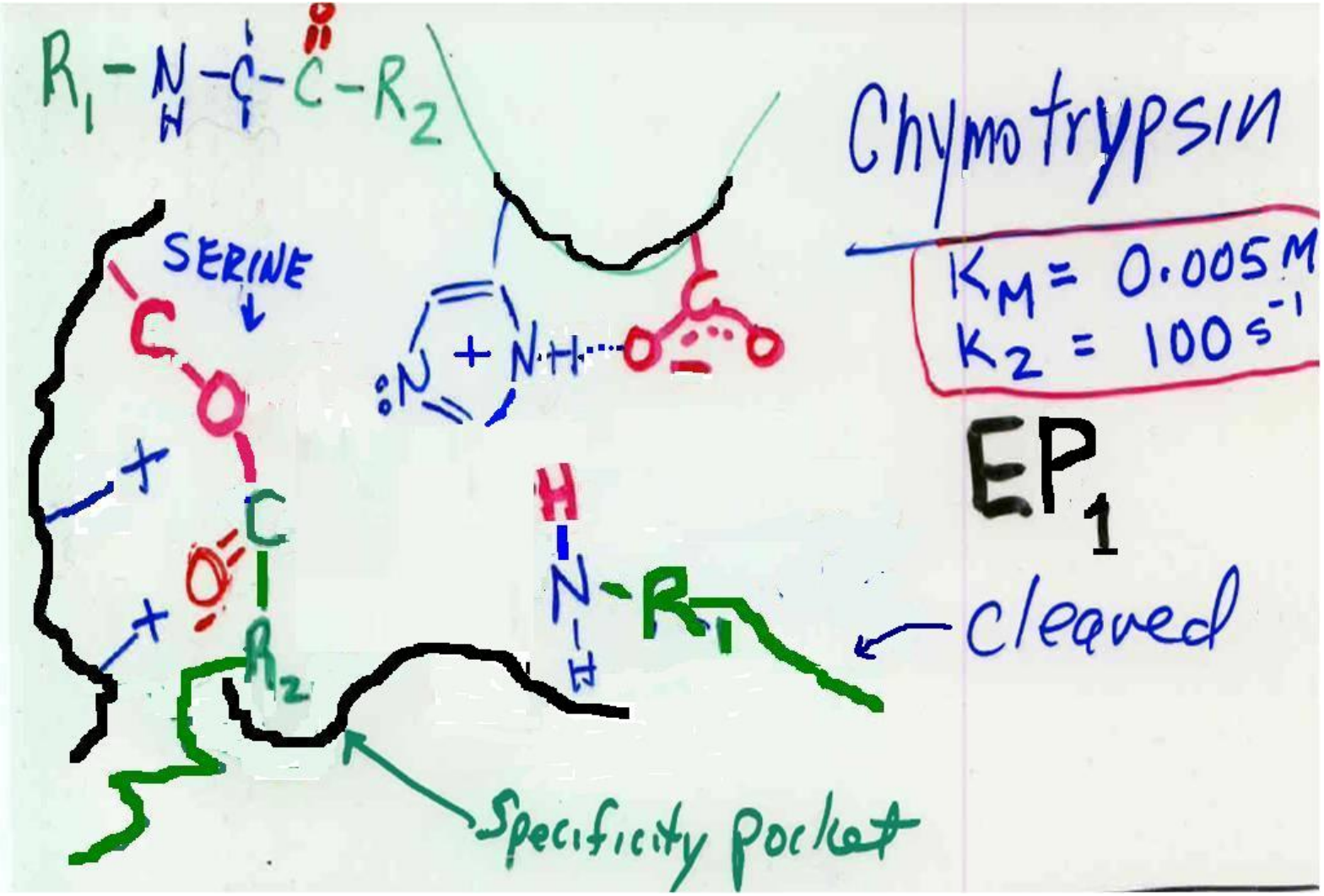
Example of electrostatic importance in an enzyme



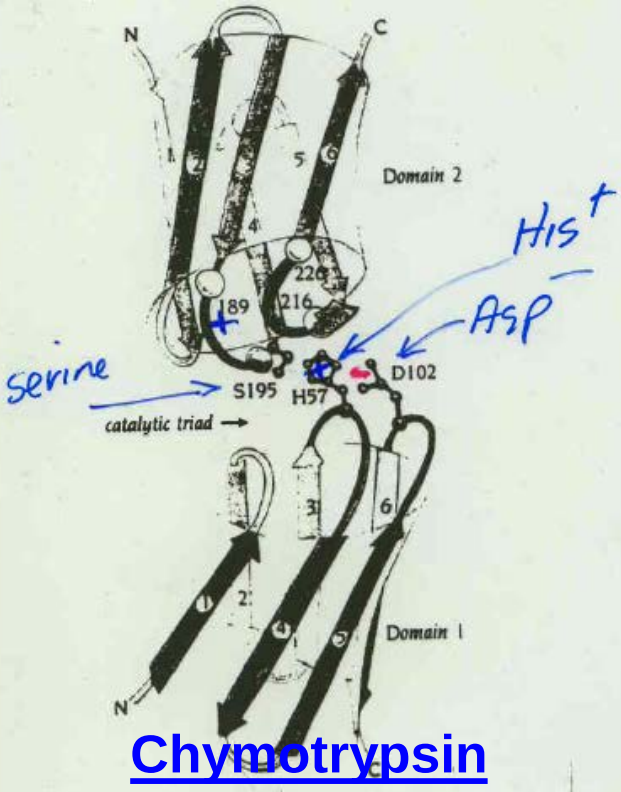

Chymotrypsin
$K_M = 0.005\,M$
$K_2 = 100\,s^{-1}$
ES_1
SERINE
protein
to be cleaved
Specificity pocket



This part of substrate is now covalently attached to the enzyme

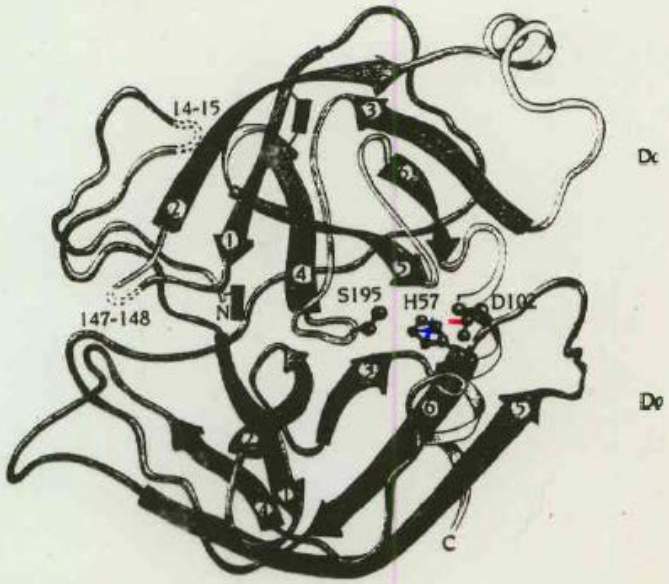

Chymotrypsin
$K_M = 0.005\,M$
$k_2 = 100\,s^{-1}$
EP_1
SERINE
cleaved
Specificity pocket

"Convergent Evolution"



Chymotrypsin

Subtilisin (bacterial)



oxyanion hole (res. 193-195)

main-chain substrate binding (res. 214-216)

substrate specificity pocket (res. 189, 216, 226)

Figure 15.10 Topological diagram of the two domains of chymotrypsin, illustrating that the essential active-site residues are part of the same two loop regions (3–4 and 5–6) of the two domains (colored red). These residues form the catalytic triad (red), the oxyanion hole (green), and the substrate binding regions (yellow and blue) including essential residues in the

Convergent evolution has produced two different serine proteinases with similar catalytic mechanisms

These four features all occur in an almost identical fashion in all members hymotrypsin superfamily of homologous enzymes, which includes a ther enzymes chymotrypsin, trypsin, elastase, and thrombin. Naively night imagine that such a combination of four characteristic features had nly once during evolution to give an ancestral molecule from which all roteinases diverged. However, subtilisin, a bacterial serine proteinase w mino acid sequence, and as we will see, a three-dimensional structure ifferent from the mammalian serine proteinases, exhibits these same haracteristic features. Subtilisin is not evolutionarily related to the chym n family of enzymes; nevertheless, the atoms in subtilisin that particip he catalytic triad, in the oxyanion hole, and in substrate binding are in a