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

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

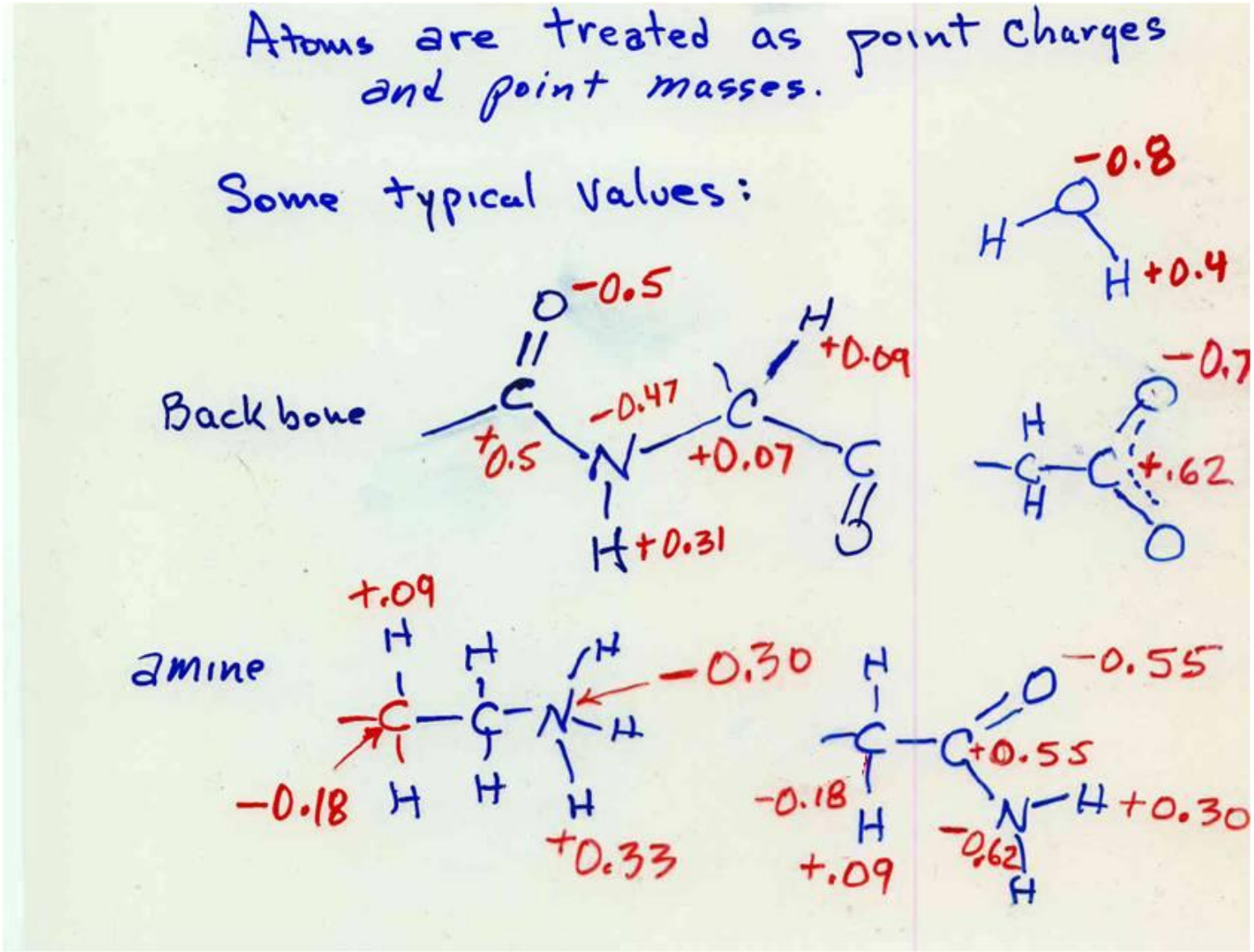
$$\text{Rate constant } k = A\, e^{\frac{-E_a}{RT}} = A10^{\frac{-E_a}{5.7kJ}}$$

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

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

$$10^{\frac{13.9}{5.7}} = 10^{2.4} = 250 \text{ times larger!}$$

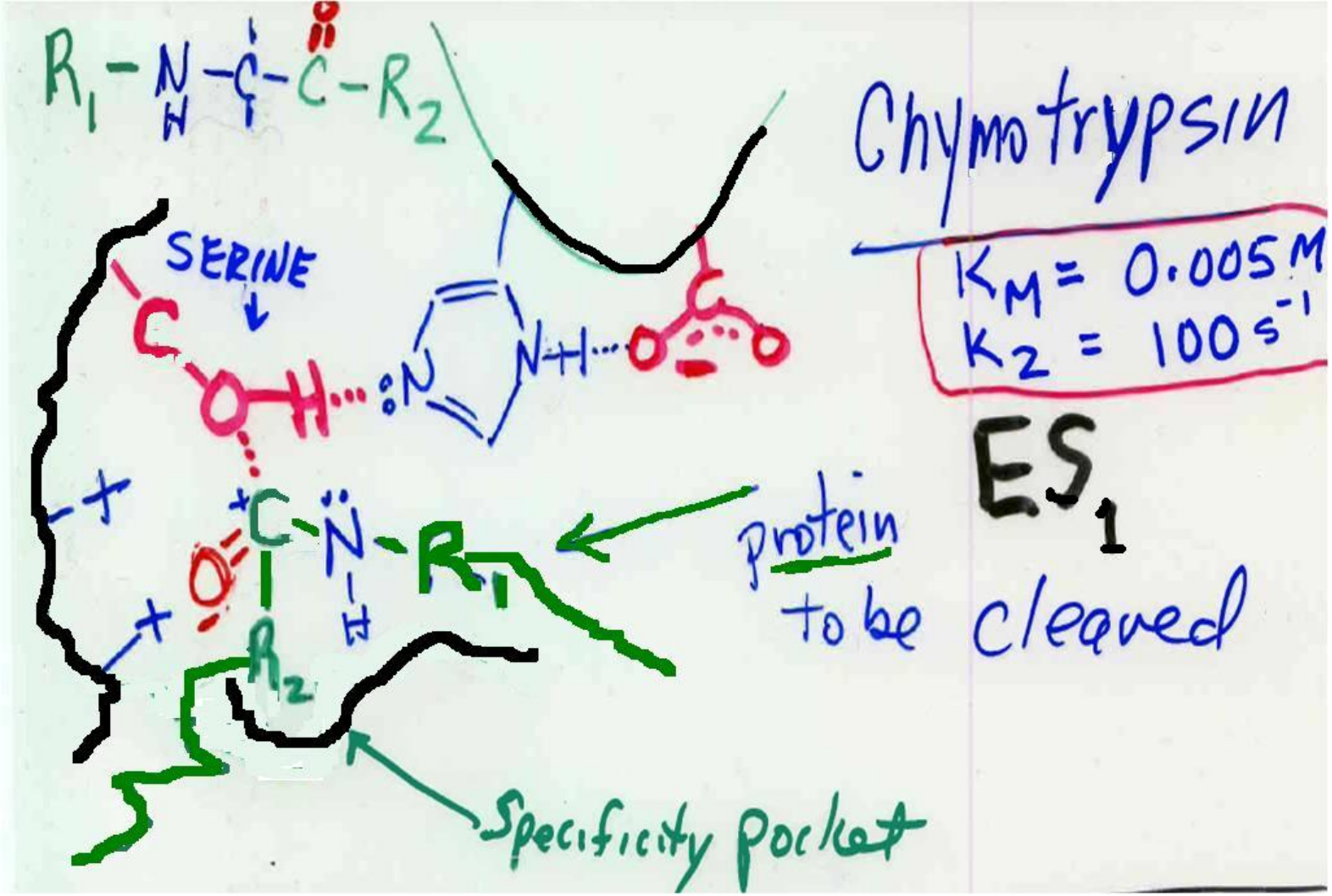
Partial Charges abound in proteins!

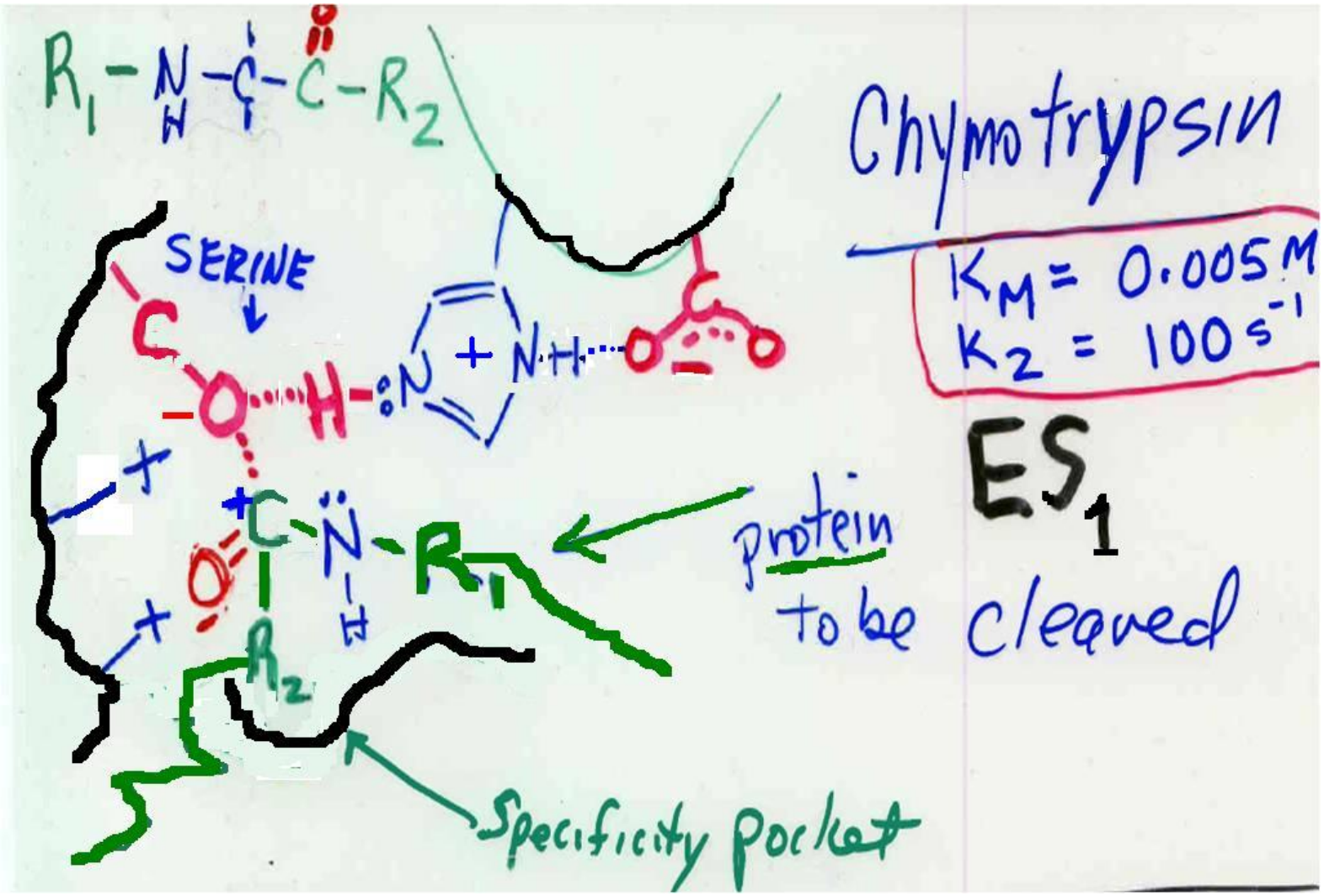


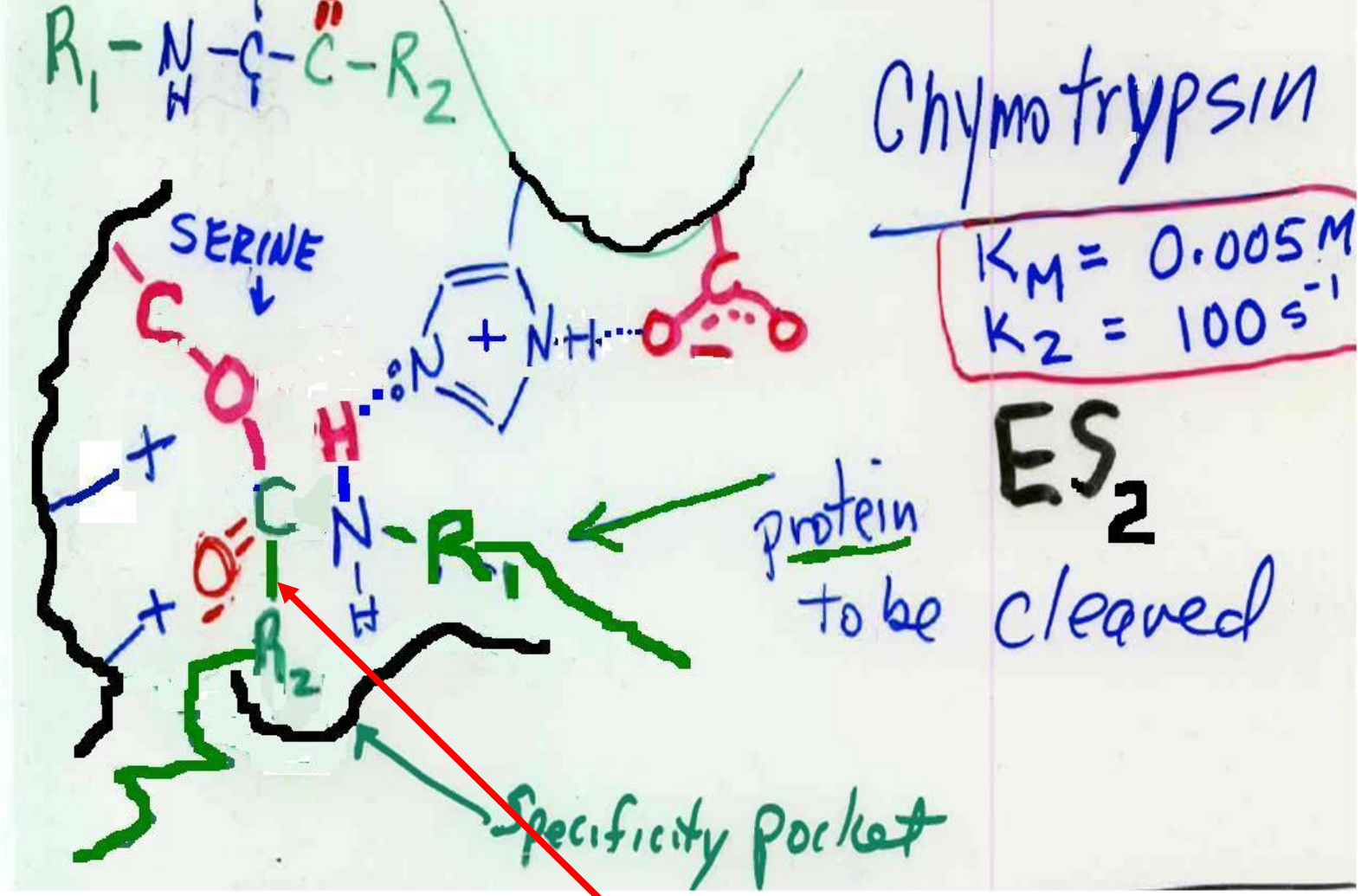
Enzymes Chymotrypsin, Subtilisin , and many othe

One of “Life’s Cutting tools”, Scissors, Knives, Saws, etc”

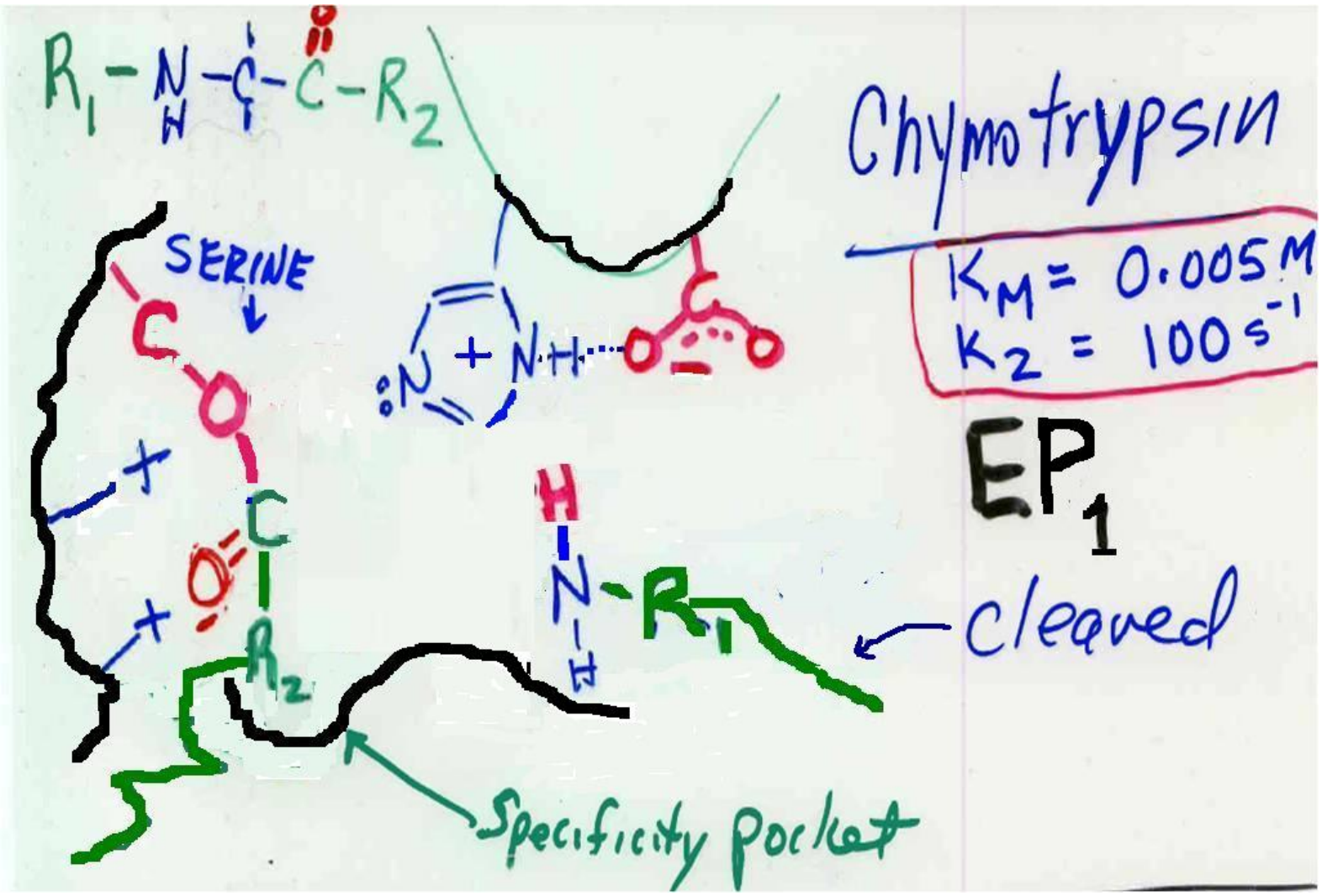
are **serine proteases**


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

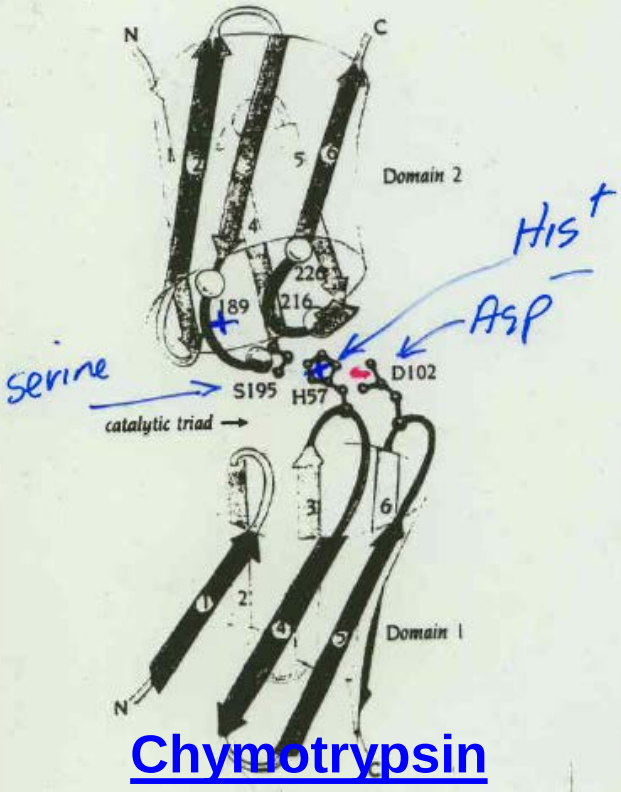

Chymotrypsin
$K_M = 0.005\ M$
$K_2 = 100\ s^{-1}$
SERINE
ES_1
protein to be cleaved
Specificity pocket



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

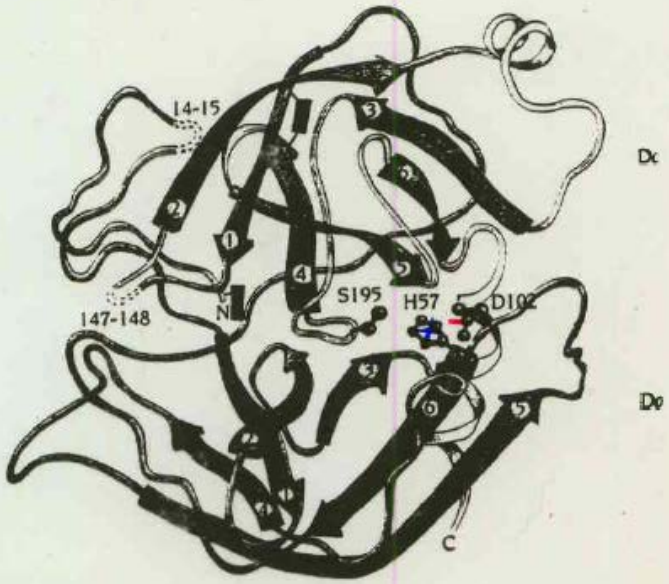
oxyanion hole (res. 193-195)

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

Subtilisin (bacterial)



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

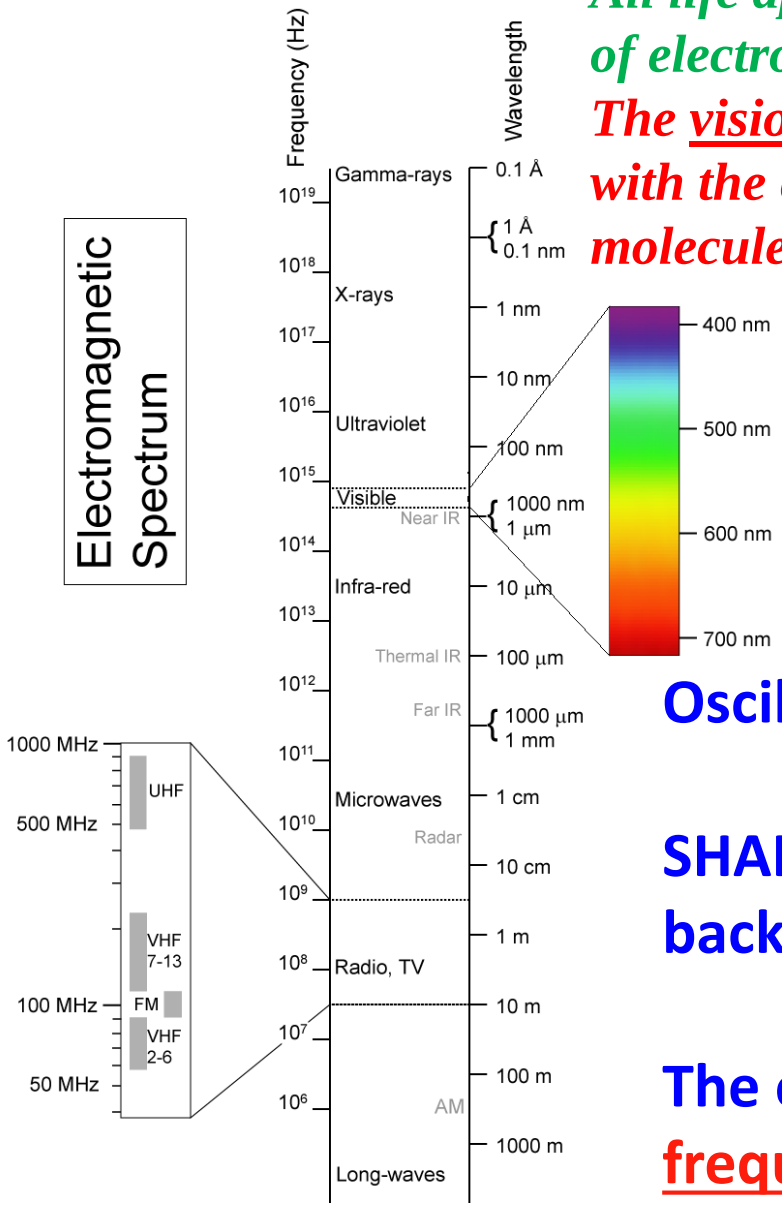
These four features all occur in an almost identical fashion in all members hymotrypsin superfamily of homologous enzymes, which includes a ther enzymes chymotrypsin, trypsin, elastase, and thrombin. Naively night imagine that such a combination of four characteristic features had nly once during evolution 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

Chymotrypsin, Subtilisin , and many others are serine proteases
In Life's Tool Kit these are the
" Scissors, Knives and Saws" for proteins.

We have often mentioned **Life's Fuel Cells i.e., Mitochondria.**

Now, we will consider the most important ingredient of the most important tool of all,

the Solar Cells: **LIGHT**



All life appears to be nurtured by the excitation of electrons by light in photosynthesis.

The vision enjoyed by higher life forms begins with the electronic excitation of a conjugated molecule within a G-protein coupled receptor.

LIGHT

Oscillating Electric and Magnetic Fields:

SHAKE charges and magnetic dipoles back and forth

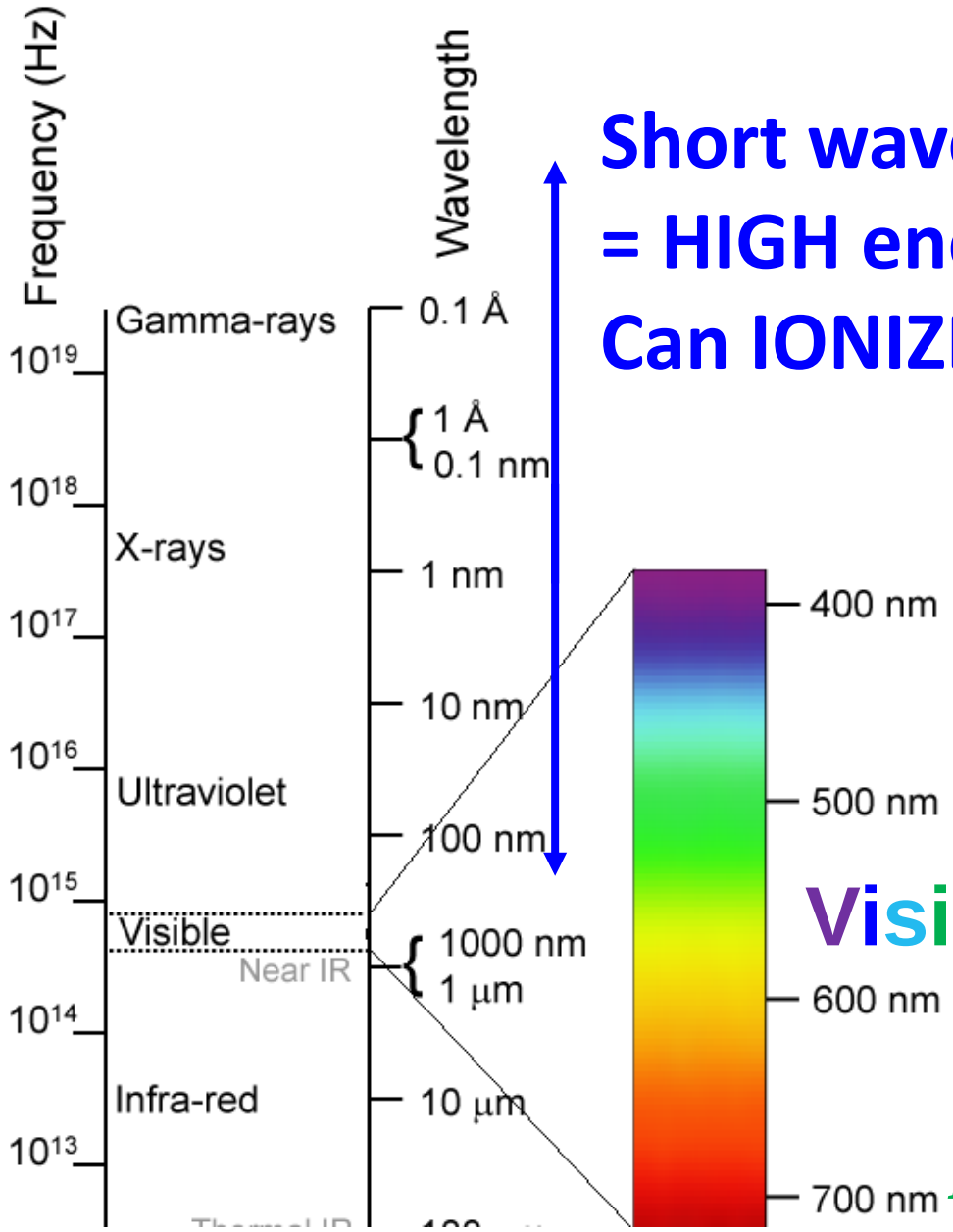
The oscillations are of all possible frequencies

Closely related to **electrophoresis**

Recall from 2 lectures ago

Force on a charge = electric field x charge
= volts/m x charge
= joules/coulomb meter x coulombs
= joules/coulomb meter x coulombs
Or energy from moving a charge

Force x distance
= Field x charge x distance
= volts/meter x charge x distance
= joules coulomb^{-1}meter^{-1} coulomb meter =joules



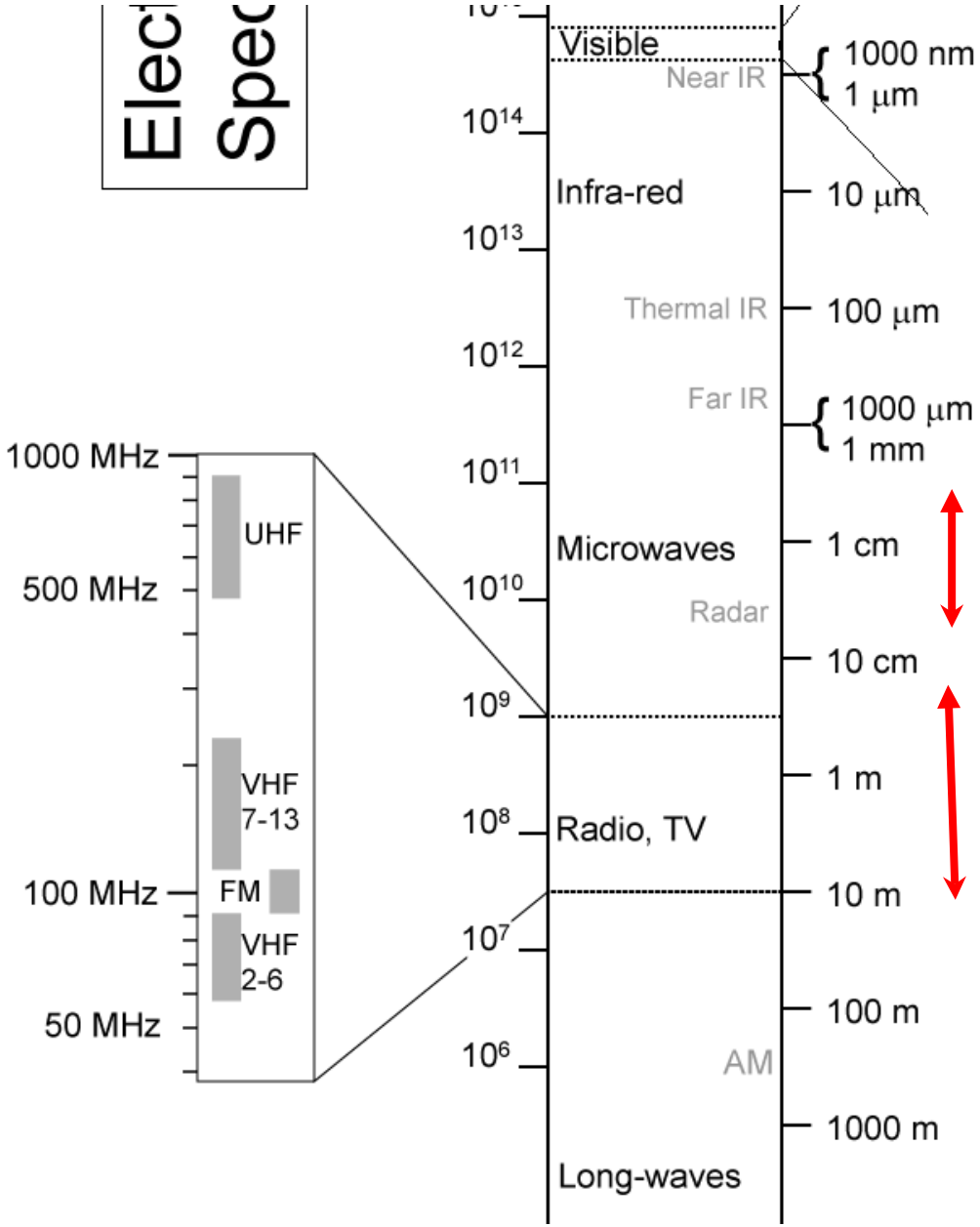
Short wavelengths
= HIGH energy photons
Can IONIZE < 250 nm

UV C <300 nm
UV B 310-290 nm
UV A 310-400 nm

Photochemistry out to 700 nm

Vision

photosynthesis.



Makes molecule VIBRATE

$\Delta E = h\nu_{vib}$

Thermal IR: WE emit this wavelength

Larger photons than microwaves

Flip electron "spin" down→"spin" up

Make molecules ROTATE

$\Delta E = h\nu_{precssion\ of\ magnetic\ dipole}$

CELL phones

Flip NUCLEAR spin

Long wavelengths

= Very LOW energy photons

treated Classically

(like translational energy of molecules)

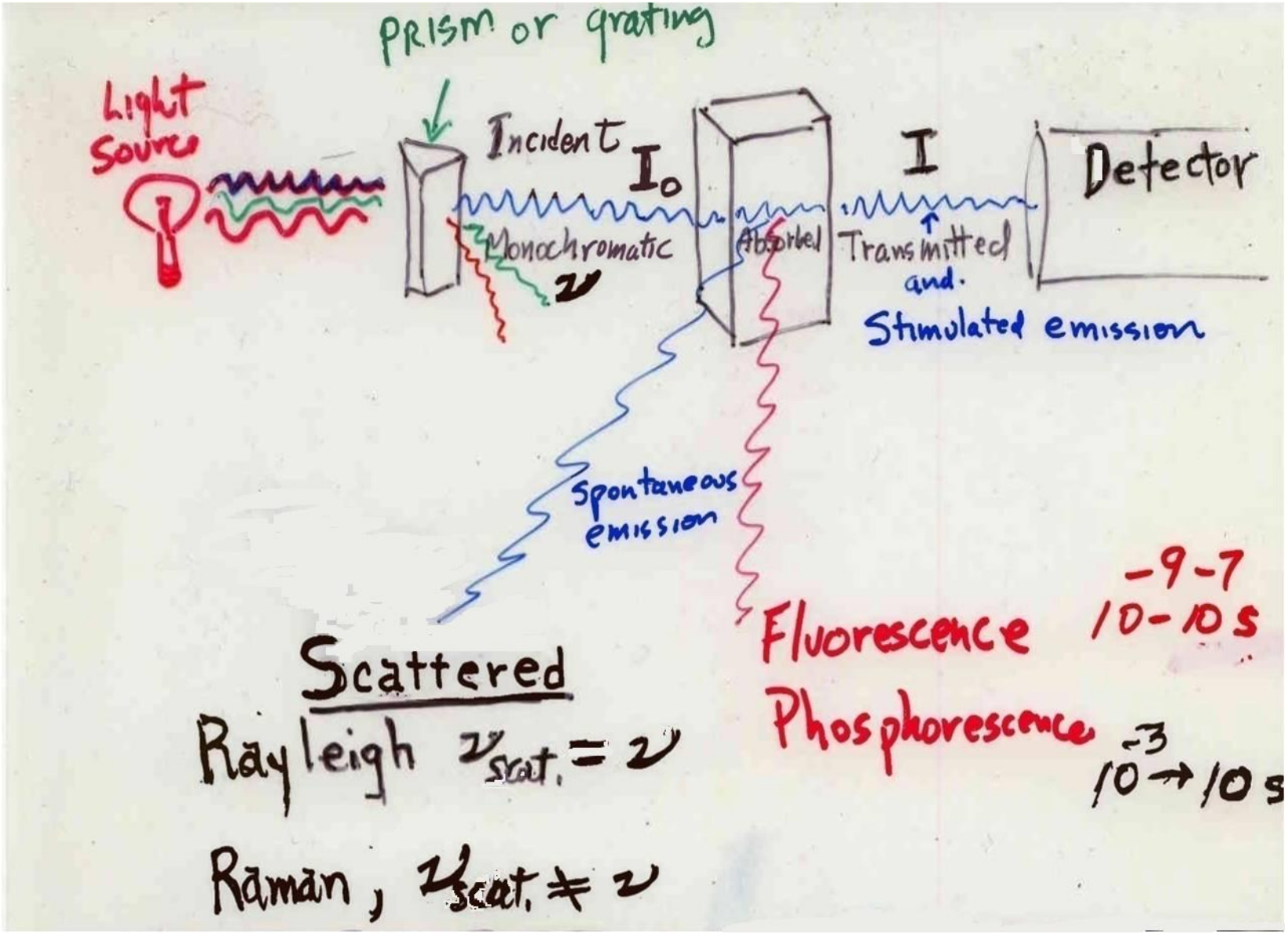
Important to realize: at 300 K (room temperature) **$\Delta E = kT$**
$= RT = h\nu$ 8.3 x 300 = 2.5 kJ/mol and
$\nu = kT/h = 1.38 \times 10^{-23} \times 300 / 6.62 \times 10^{-34} =$

$\Delta E = h\nu_{vib}$

EVERY **THING at 300 K**
(including you and I) is **emitting 0.005 cm = near 50 micrometers**

In other words we all emit microwaves because of our temperature.

At around 300K , ΔE is close to kT


PRISM or grating
Light Source
Incident
I_o
Monochromatic
ν
Absorbed
I
Transmitted and Stimulated emission
Detector
Spontaneous emission
Fluorescence
10^{-9} - 10^{-7} s
Phosphorescence
10^{-3} → 10 s
Scattered
Rayleigh $\nu_{scat.} = \nu$
Raman, $\nu_{scat.} \neq \nu$

What is Absorbance?

Beer-Lambert Law ($A = \varepsilon cx$)

photon + M ---> M* (electronically excited molecule)

$$\frac{d[photon]}{dt} = -k[M][photon] = -k\,[M]\,I$$

$[photon]$ is proportional to light intensity $= I$

$$\frac{dI}{dt} = -k[M]I \text{, where } k[M] \text{ is a } \textit{pseudo first-order rate constant}$$

for the disappearance of photons (time, $t = x/\text{speed of light}$)

$$f = \frac{I}{I_0} = e^{-k[M]t} = e^{-k[M]x/c} = 10^{\frac{-k[M]x}{2.303c}} = 10^{-\varepsilon[M]x} = 10^{-A}$$

In this context **f = fraction of photons remaining after travelling distance x**

i.e., **f= Transmittance = T = $10^{-\varepsilon cx} = 10^{-A}$**

10^{-A} is just telling you that **$A = \varepsilon cx = -\log T$**

These two are <u>EXACTLY</u> the <u>SAME</u> thing. Learn and use <u>BOTH</u>

Beer-Lambert Law ($A = \varepsilon cx$)

suppose **A = 2** What fraction of light is transmitted?

$10^{-2} = 0.01 = 1\ \%$

Now, double the concentration.
What fraction of light is transmitted?

$10^{-4} = 0.0001 = 0.01\ \%$

Now, double the path length using this concentration.
What fraction of light is transmitted?

$10^{-8} = 10^{-6}\ \%$

Now, change the wavelength until ε is doubled.
What fraction of light is transmitted at this wavelength?

$10^{-16} = 10^{-14}\ \%$

UV absorption of Amino Acids

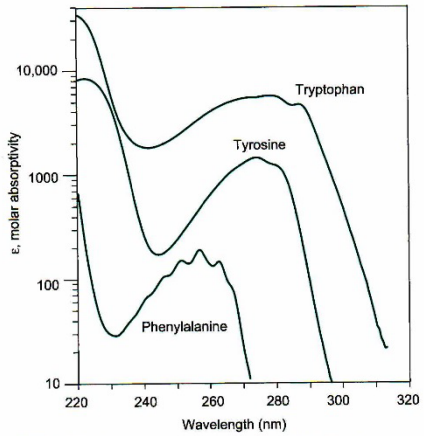


FIGURE 13.15 Absorption spectra of the aromatic amino acids (tryptophan, tyrosine, and phenylalanine) at pH 6.

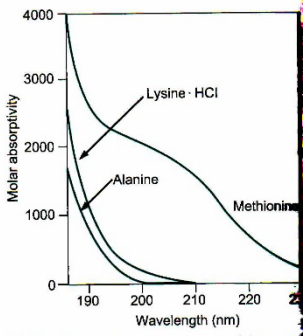
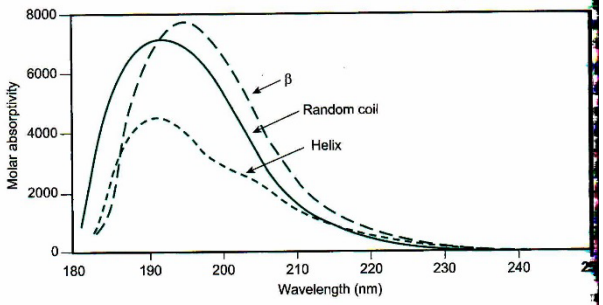


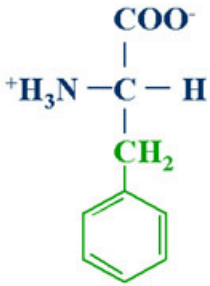
FIGURE 13.16 UV-absorption spectra of three α-amino acids in aqueous solution at pH 5.

Polypeptide Spectra

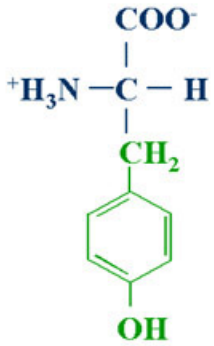
The contribution of the amide linkages to the absorption spectra can be see by comparing the spectrum of lysine hydrochloride in figure 13.16 with that poly-L-lysine hydrochloride in the random-coil form (figure 13.17). The broad absorption centered at 192 nm ($\varepsilon_{192} = 7100\ M^{-1}\ cm^{-1}$) is characteristic of the amide linkage in poly-L-lysine and increases the absorbance in this region by eightfold over that the free amino acid. All proteins have contributions to the absorption spectra in the region around 190 nm (180 to 200 nm) from the polypeptide backbone; however, the are accompanied by absorption contributions from certain of the side chains, especially the aromatic ones.

FIGURE 13.17 UV-absorption spectra of poly-L-lysine hydrochloride in aqueous solution; random coil, pH 6.0, 25°C; helix, pH 10.8, 25°C; β form, pH 10.8, 52°C.
(K. Rosenheck and P. Doty, The Far Ultraviolet Absorption Spectra of Polypeptide and Protein Solutions and Their Dependence on Conformation PNAS 1961 47 (11) 1775–1785. Reprinted by permission of the Estate of Paul M. Doty.)

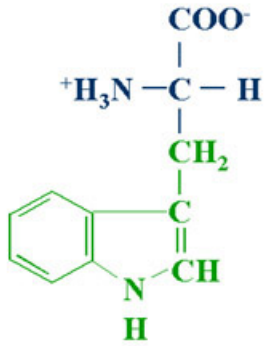




Phenylalanine



Tyrosine



Tryptophan