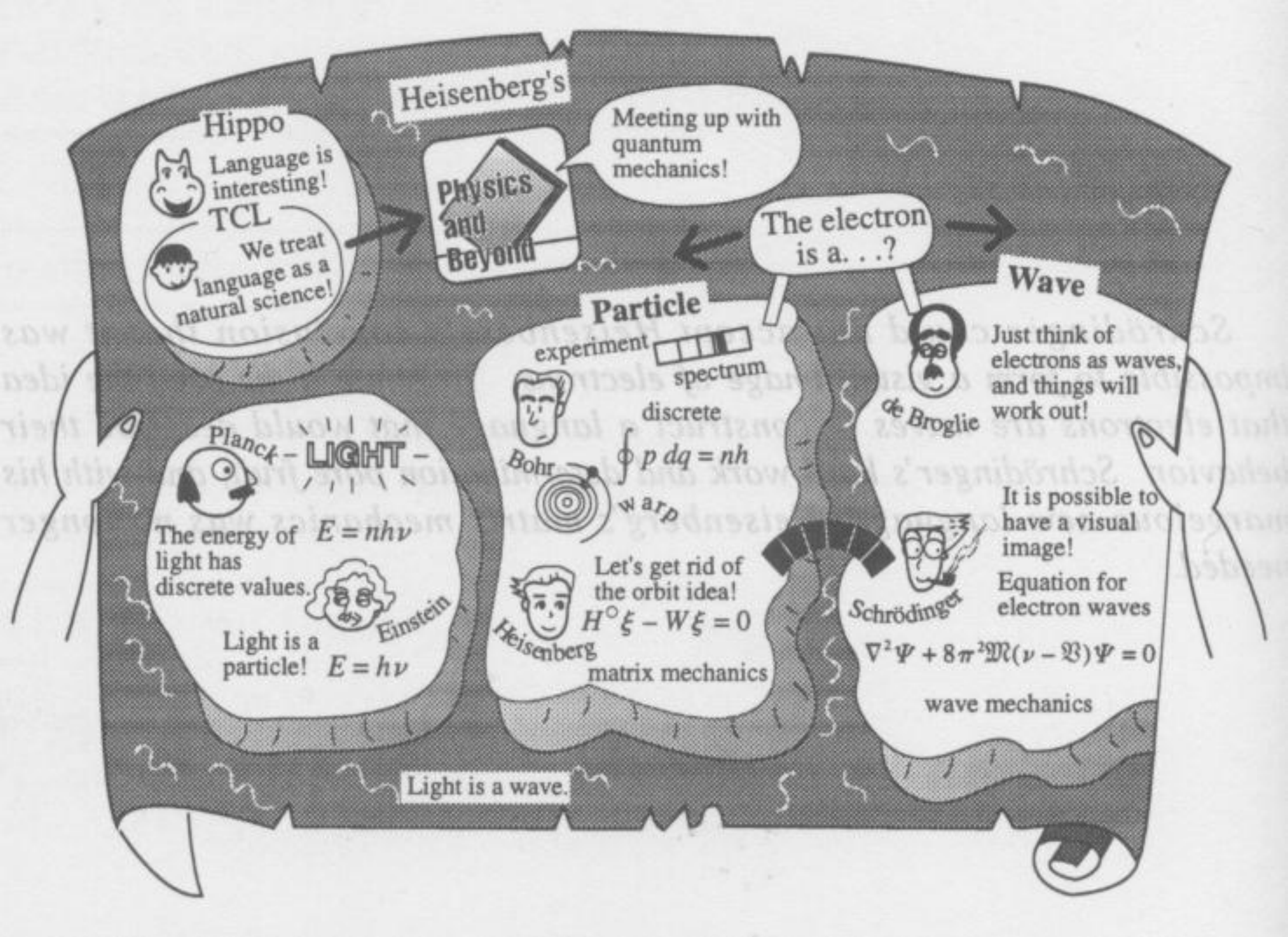
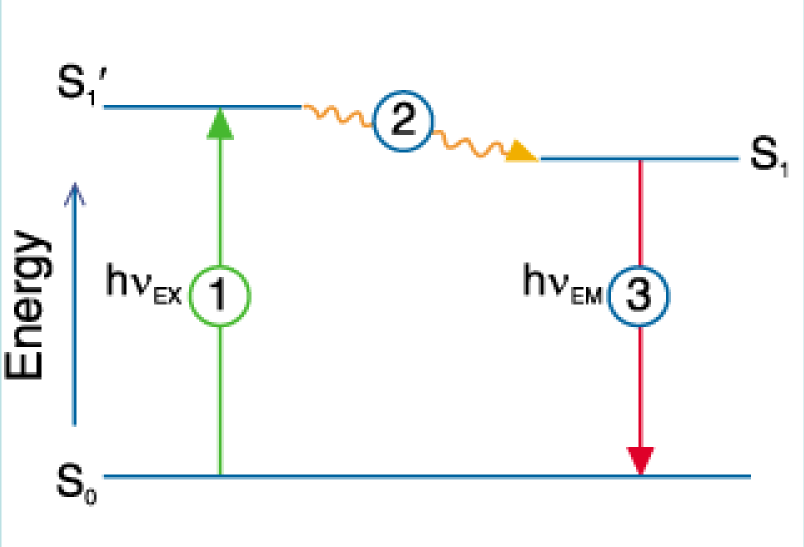
How do you describe what a molecule does in an excited state?

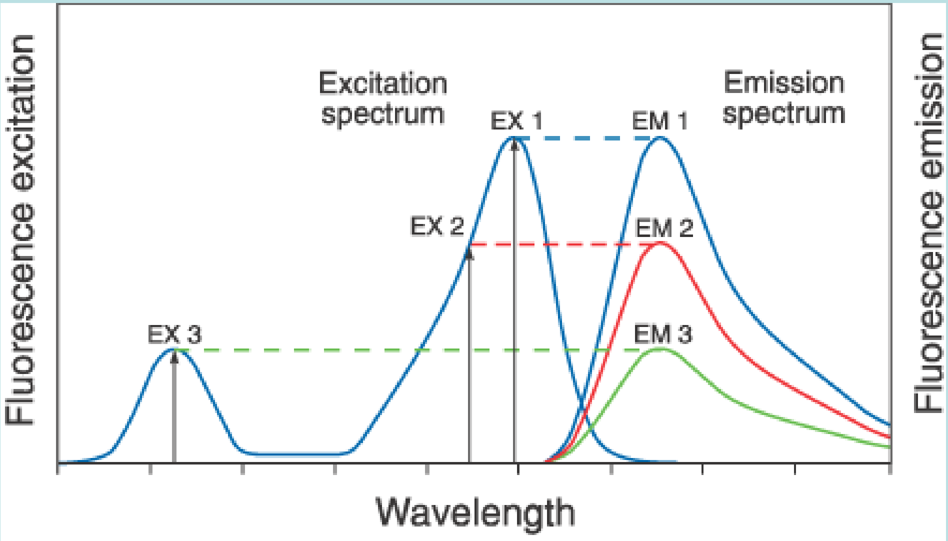
In general we would get serious about quantum mechanics

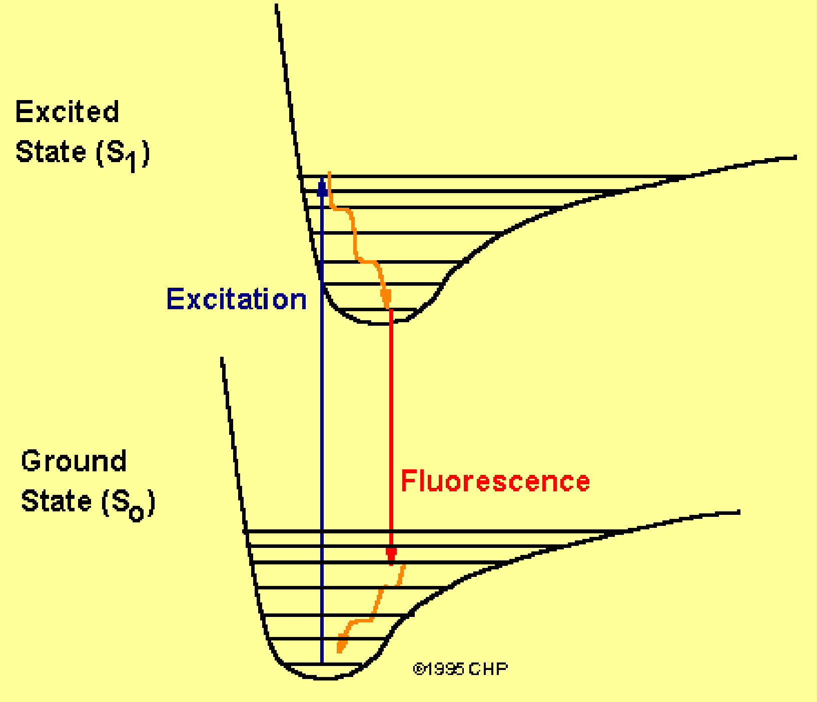


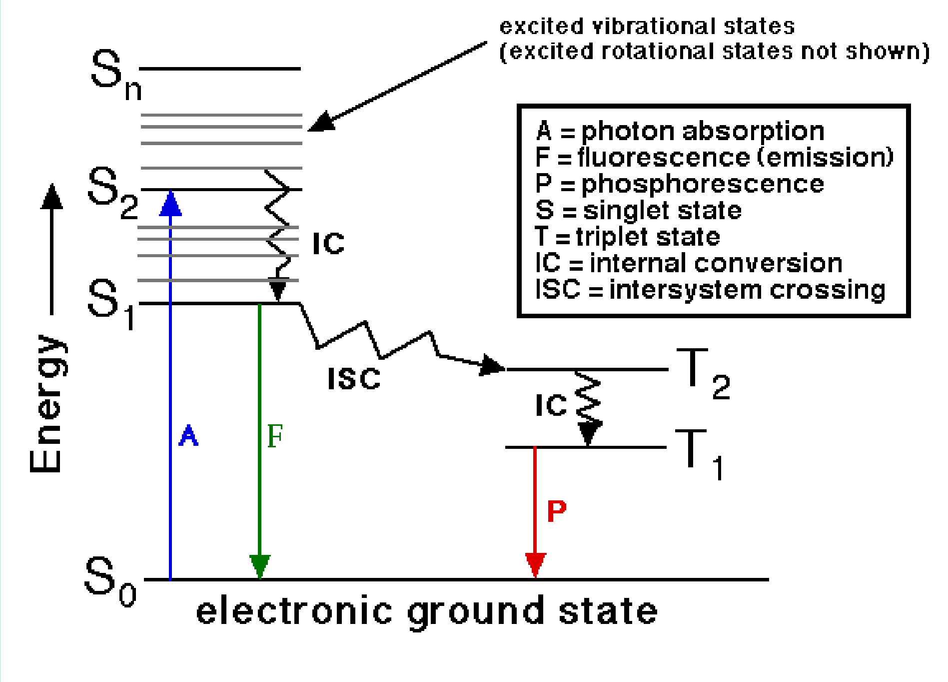
From “What is Quantum Mechanics? A Physics Adventure, Transnational College of LEX

Usual description of fluorescence

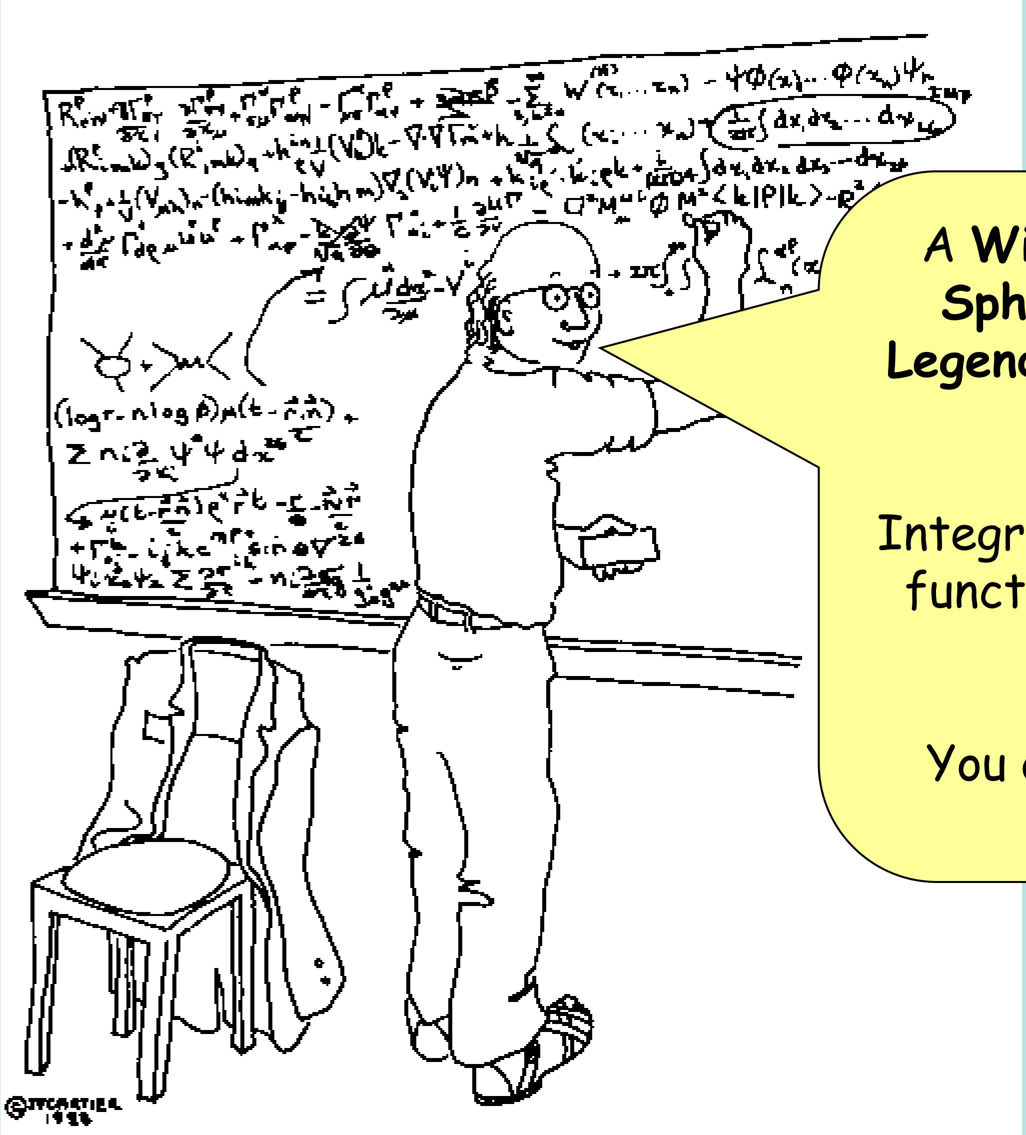








A = photon absorption
F = fluorescence (emission)
P = phosphorescence
S = singlet state
T = triplet state
IC = internal conversion
ISC = intersystem crossing



"At this point we notice that this equation is beautifully simplified if we assume that space–time has 92 dimensions."

A **Wigner Rotation Matrix** here, a **Spherical Harmonic** there, and a **Legendre Polynomial** everywhere, and you have the answer.
Got it?
Integrate a bit, expand in these lovely functions, orthogonalize everything, and so on.
It's easy – no sweat.
You only need 5 years of graduate school to understand!

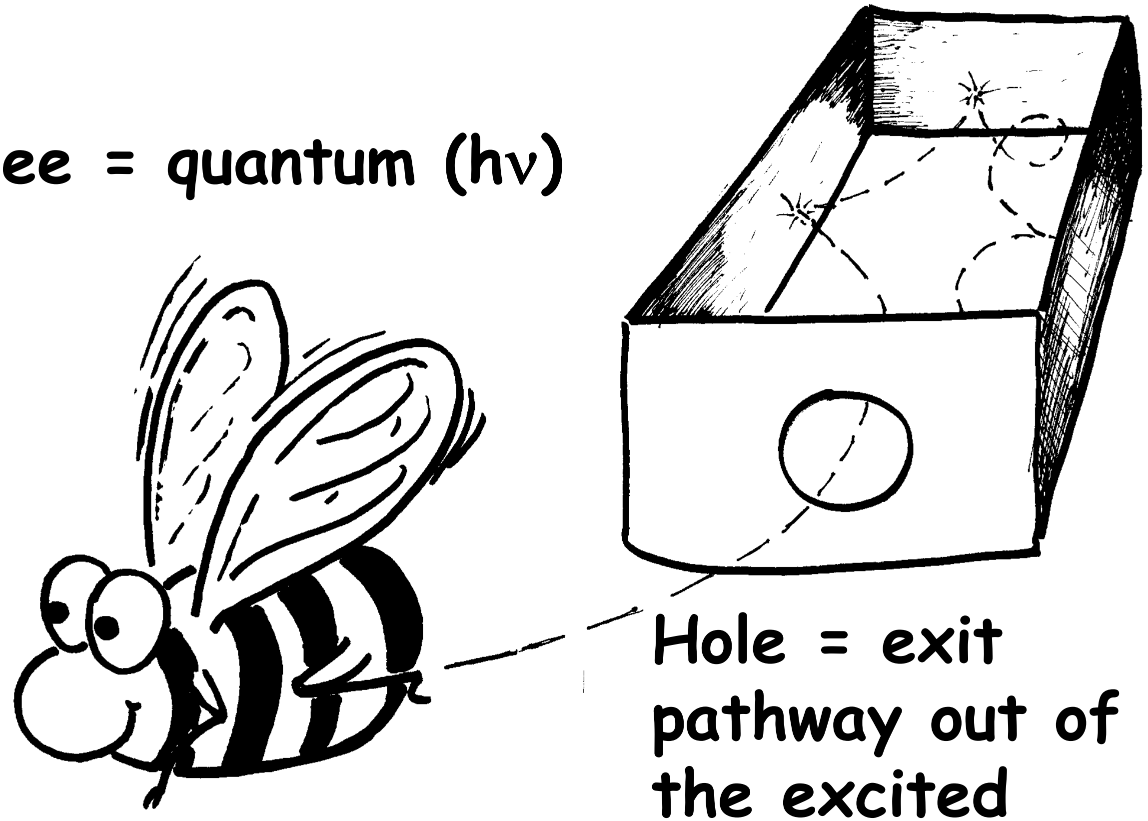
A theoretician solving problems of spectroscopy

We don't need all those complications to understand some basics of fluorescence

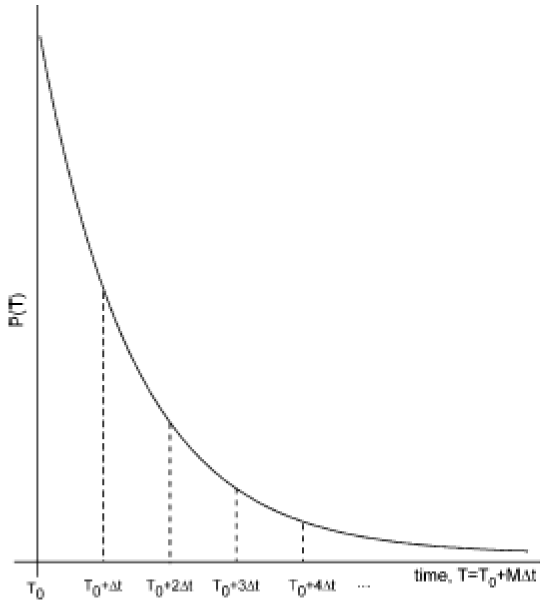
Analogy for spontaneous emission

Box = excited state

Bee = quantum (hν)



Hole = exit pathway out of the excited state



A plot of the probability that a bee (Fig. 5) will still be in a box after time $T = T_0$? M Dt. If we assume that $T_0 = 0$ (starting time), then $P(T) = \exp(-k_f T)$. P(T) is identical to the probability that a single molecule will still be in the excited state after time T has passed if it became excited at time $T_0 = 0$. See the text for the derivation, and for the extension to multiple holes (pathways of deexcitation) and to an ensemble of bees (molecules) in the box (excited state)

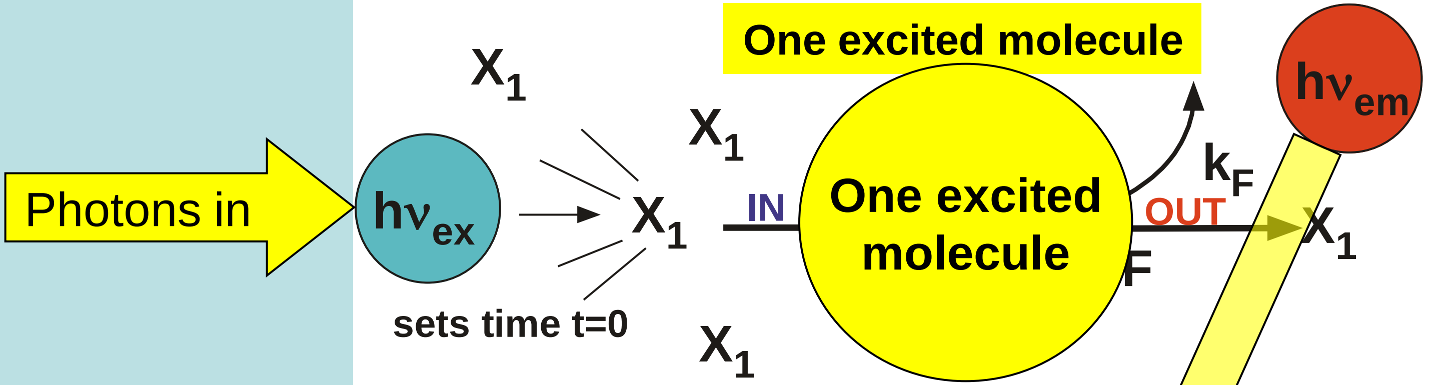
MOLECULE IN THE EXCITED STATE

- *some statistics* -

Simple case: one door in, and one door out,
one molecule occupation,
molecule has no memory

Emission
of a photon

EXCITED STATE ROOM



The emission
Is a random
process

k_F (a constant!) is the probability per unit time that X_1* will exit through door F

probability that X_1* is still in the excited-state room at time t is:

$$e^{-k_F t} = e^{-t/\tau_F}$$

The probability
of the emission
of a photon is
an exponential

Proof: that $e^{-k_F T}$ is the probability that X* is in the room at time t=T.

1.) k_F is the probability ***per unit time*** that X*, which is already in the excited state at any time "t", will **leave** the excited state through door "F" (fluorescence).

(1.1) $\mathbf{k_F \Delta t}$ is the *approximate* probability that X*, which is already in the excited state at time "t=0", will **leave** the excited state through door "F", **after the "short" time, Δt**.

2.) (1-k_F) is the probability *per unit time* that X*, which is already in the excited state at any time "t", will **remain** in the excited state.

(2.1) **$(1-k_F\Delta t)$** is the *approximate* probability that X*, which is already in the excited state at time "t=0", will **remain** in the excited state for the "short" time Δt.

(2.1.1) another Δt time step: **$(1-k_F\Delta t)(1-k_F\Delta t)$** is the *approximate* probability that X*, which is already in the excited state at time "t=0", will **remain** in the excited state for the time 2Δt.

… **and so on for 3Δt, etc.**

3.) Consider the total time from t=0 to t=T. Divide up this time interval into "n" time points, so that **$\Delta t = T/n$**. The more time intervals, the smaller Δt.

(3.1) **$(1-k_F\Delta t)^n = (1-k_F T/n)^n$** is the *approximate* probability that X*, which is already in the excited state at time "t=0", will **remain** in the excited state for the longer time **$T = n\Delta t = n(T/n)$**.

(3.2) Take the limit as **n - >infinity**. This is the ***definition of an exponential***.

The exact probability that X* remains in the excited state until t=T is:

$$\exp(-k_F t)_{t=T}$$

QED

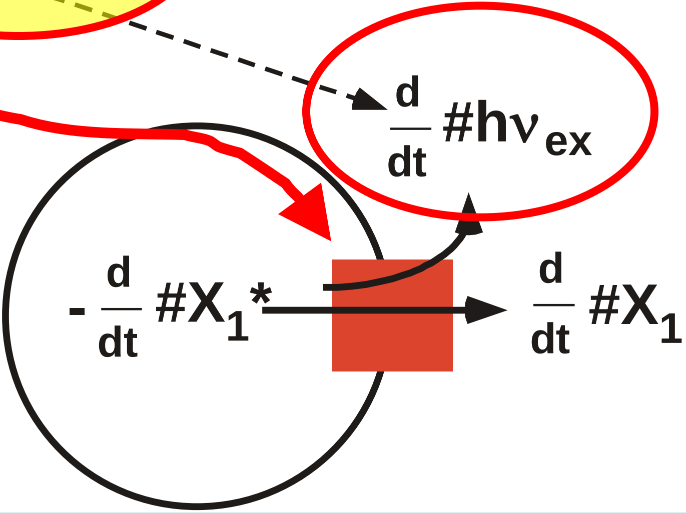
Every molecule exiting through the door "F" loses (emits) a photon $h\nu_{ex}$

X_1* molecules exiting per unit time through door F = $[\#X_1^*]k_F$; so $d[\#X_1^*]/dt = -[\#X_1^*]k_F$

The Signal = emitted photons

$$d[\#h\nu_{ex}]/dt = k_F[\#X_1^*]$$

THIS IS THE INTENSITY OF FLUORESCENCE LIG



The intensity is the *number of photons* per unit time;
for a single molecule it is proportional to the rate of leaving

But this does not tell us much except that the molecule is there.

MOLECULE IN THE EXCITED STATE

- *some statistics* -

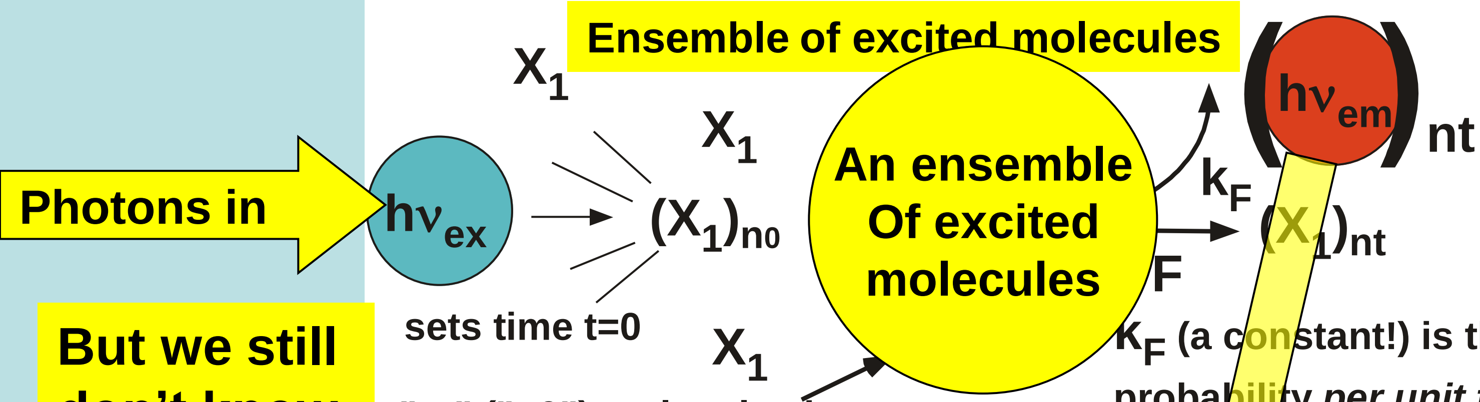
Now let's look at an ensemble of molecules

More complex case: one door in, and one door out,
multiple molecule occupation,
molecules have no memory

EXCITED STATE ROOM

all X_1*molecules are identical and act independently

Emission of photons



sets time t=0

"nt" ("n0") molecules in excited-state room at time "t" ("t"=0)

k_F (a constant!) is the probability *per unit time* that any X_1* molecule will exit through door F

But we still don't know much except that the molecules are there. Not much information

The emission from an ensemble is still an exponential

# of molecules still in excited-state at time "t" is:

$$[\#X_t^*] = [\#X_0^*]e^{-k_F t} = [\#X_0^*]e^{-t/\tau_F}$$

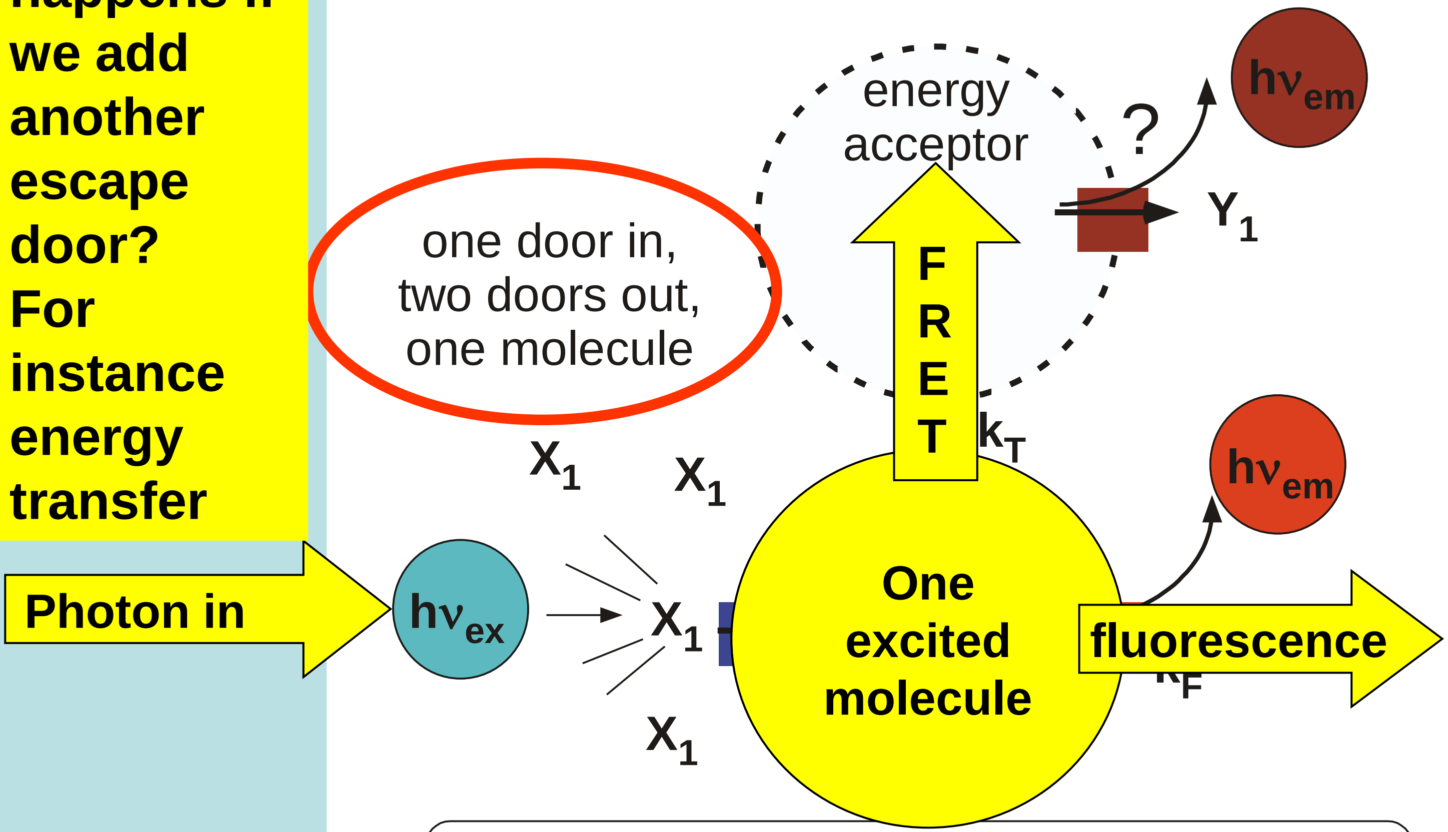
\# X_1^* molecules exiting per unit time through door F = $[\#X_1^*]k_F$; so $d[\#X_1^*]/dt = -[\#X_1^*]k_F$

But we are not really interested in the photons or the fluorescence mechanisms themselves. We are interested in all the other processes that happen in the environment of the fluorophore!

NOW TWO EXIT DOORS TO EXIT (F AND T)

F = FLUORESCENCE; T = TRANSFER

What happens if we add another escape door? For instance energy transfer



So, now we have more information. For instance we can see FRET – <u>the close vicinity of the “donor” and the “acceptor”</u>

probability *per unit time* that X_1* will exit through either door F <u>or</u> door T is : $k_T + k_F$ (a constant!)

Still get an exponential decay – but faster!

overall probability that X_1*is still in the excited-state room at time t is:

$$e^{-(k_T+k_F)t} = e^{-t/\tau_F}$$

probability that X_1*will go out door F ***eventually*** is:

$$\frac{k_F}{k_F + k_T} = \text{QUANTUM YIELD}$$

Probability of emission has decreased

The probability /time for going through the fluorescence door is always the same!

The total rate of leaving the excited state is
GREATER.

$$\textit{QuantumYield of fluorescence} = \frac{\text{total number of photons emitted}}{\text{total number of molecules originally excited}}$$

$$= \frac{k_F}{\left(\sum_{i \neq F} k_i + k_F\right)}$$

We can measure the efficiency of energy transfer from JUST the fluorescence lifetimes

$$\text{Quantum Yield of energy transfer} = \frac{\text{total number of energy quanta transferred}}{\text{total number of molecules originally excited}}$$

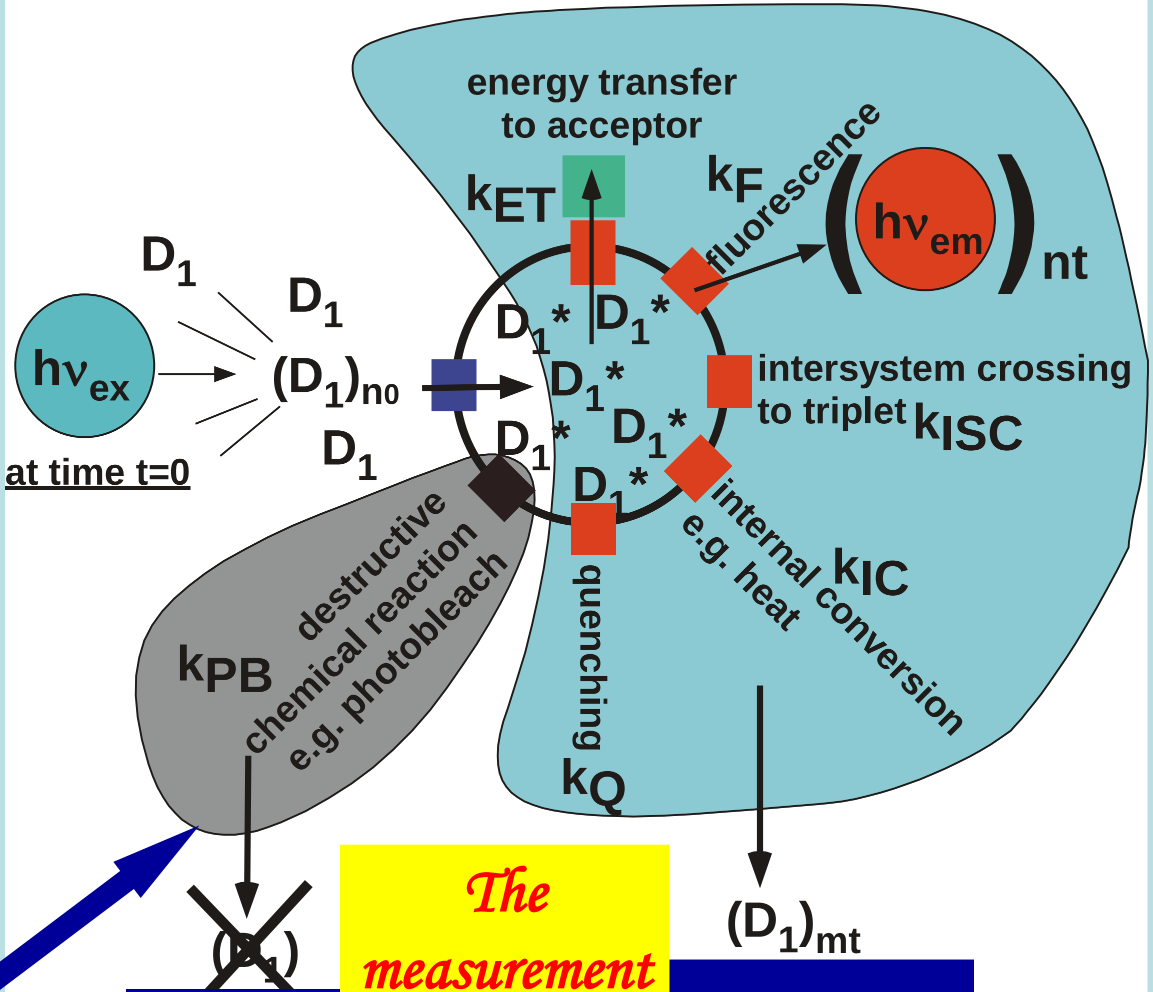
$$= \frac{k_{Transfer}}{(k_{Transfer} + k_F)} = \frac{\left(1/\tau_{+transfer}\right) - \left(1/\tau_{-transfer}\right)}{\left(1/\tau_{+transfer}\right)} = 1 - \left(\frac{\tau_{+transfer}}{\tau_{-transfer}}\right)$$

The **quantum yield** **of energy transfer** is the fraction of excited molecules that transfer a quantum of energy to the acceptor (Y)

MANY PHOTONS ($h\nu_{ex}$ & $h\nu_{em}$) AND MANY ESCAPE DOORS

The rate of leaving any of the doors can be used to measure the rate of leaving any OTHER door

In particular the photolysis, in the <u>second to minute scale</u>, can be used to measure the <u>nanosecond</u> scale exit rates



Seconds to Minutes

The measurement

Nanoseconds

the excite

$k_F + k_{ISC} + k$

Quantum yield of the i^{th} process = $\frac{k_i}{\sum_j k_j}$

Determining rate of process “p” by measuring the rate of process “m”

Rate of deactivation (1/τ) and Q.Y. of process “m” in the *presence* of *all* paths of deactivation (measuring process “m”):

$$\frac{1}{\tau_m} = \sum_j k_j \;;\; (Q.Y.)_m = \frac{k_m}{\sum_j k_j}$$

Rate of deactivation (1/t) in the *absence* of path “p” of deactivation:

$$\left(\frac{1}{\tau_m}\right)_{\neq p} = \sum_{j\neq p} k_j ;\; (Q.Y.)_{m;\neq p} = \frac{k_m}{\sum_{j\neq p} k_j}$$

Combine the two rates and quantum yield measurements

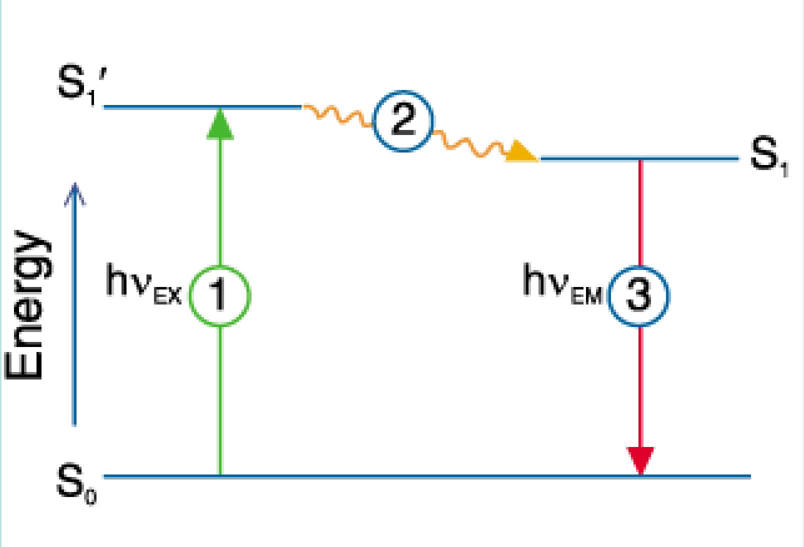
$$\frac{1}{\tau_m} - \left(\frac{1}{\tau_m}\right)_{\neq p} = k_p \;;$$

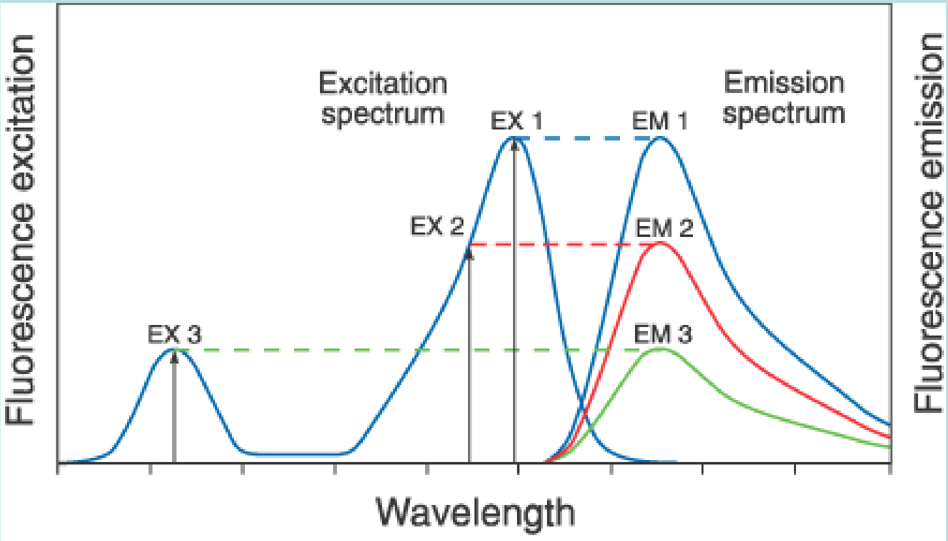
“p” can be FRET

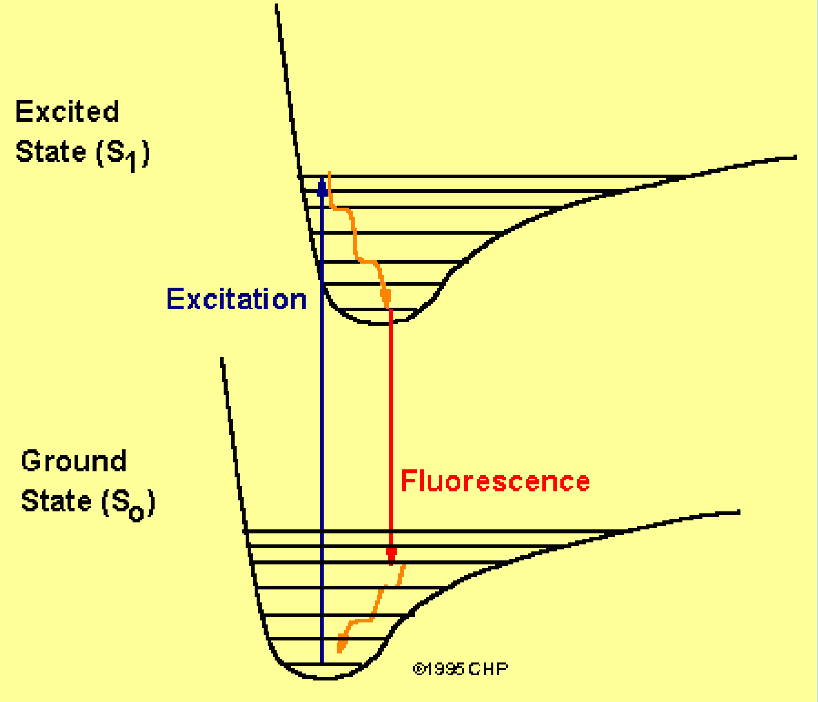
$$\frac{(Q.Y.)_m^{-1} - (Q.Y.)_{m;\neq p}^{-1}}{(Q.Y.)_m^{-1}} = \frac{k_p}{\sum_j k_j} = (Q.Y.)_p$$

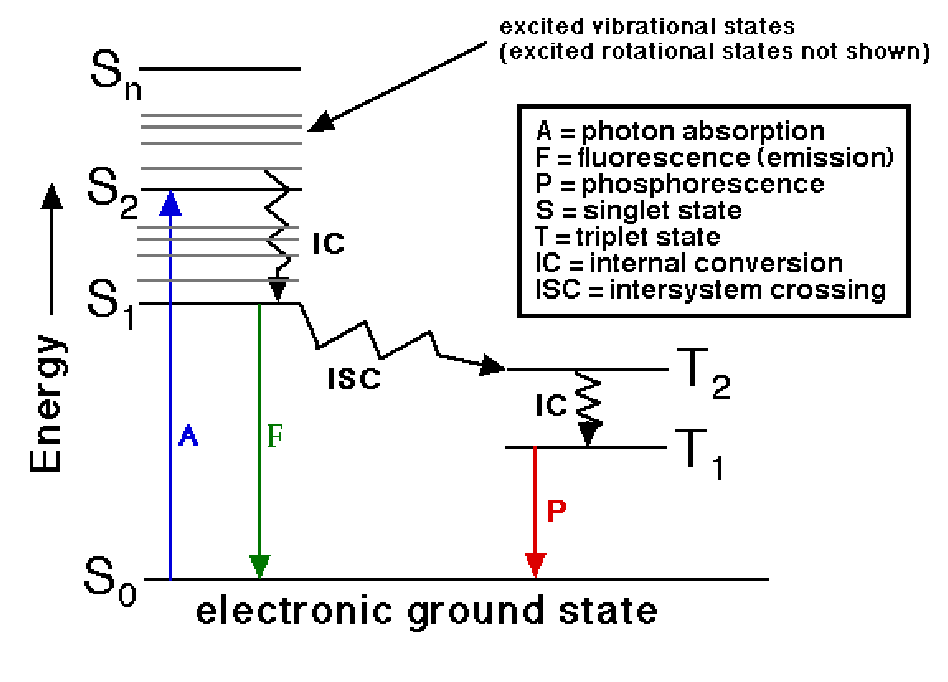
So, we can determine “p” by measuring “m”

Now let's go back to some details



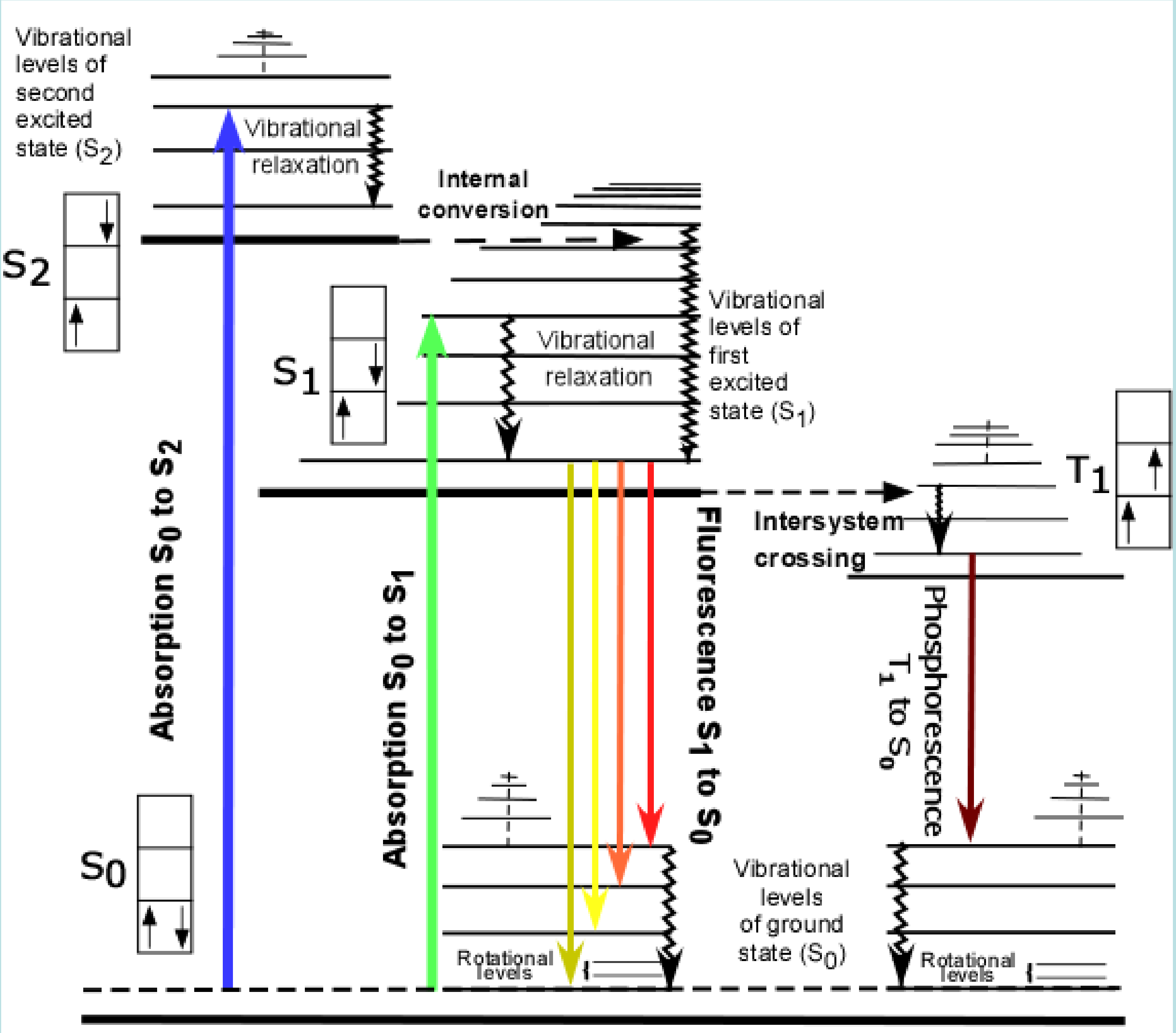


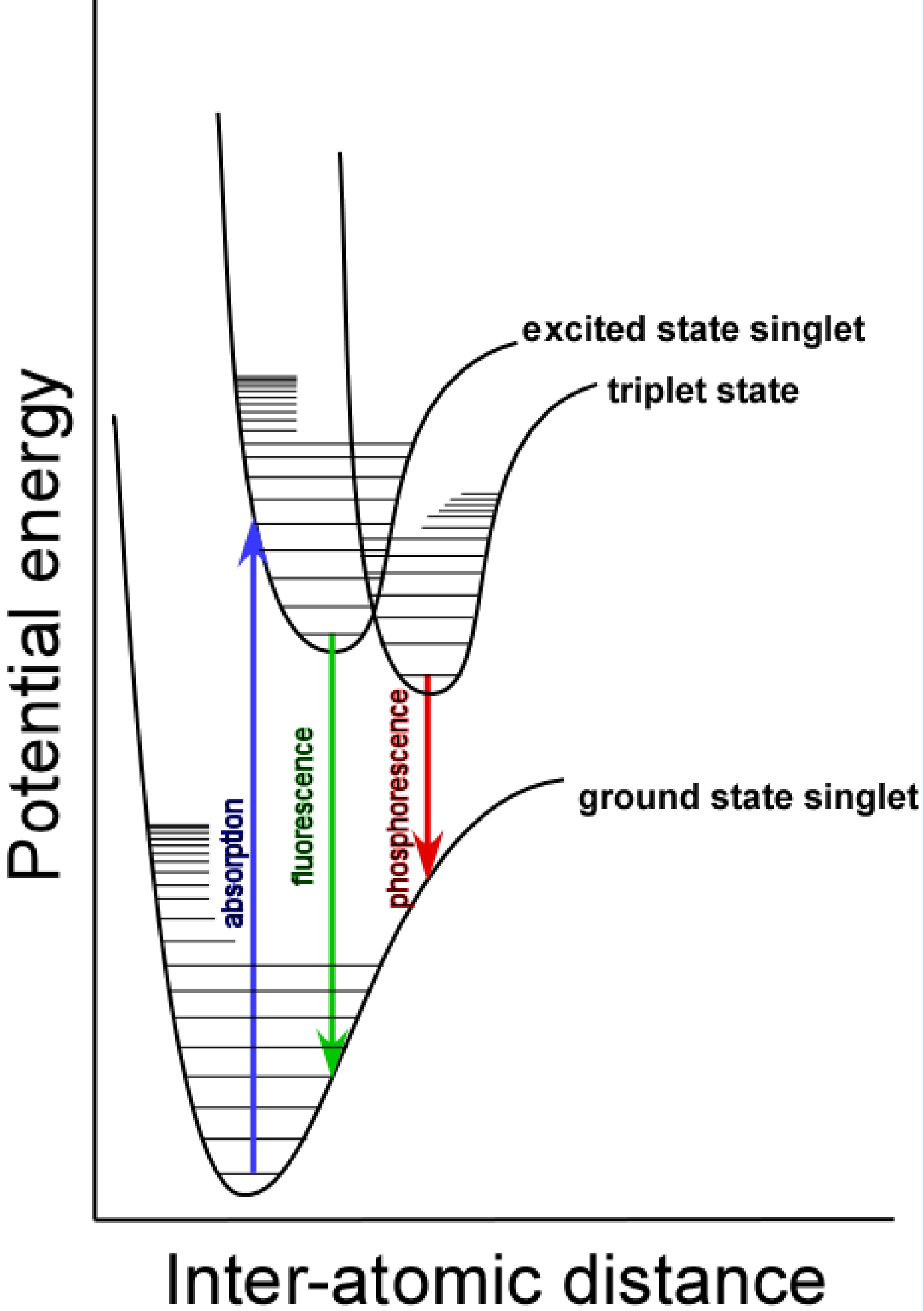




A = photon absorption
F = fluorescence (emission)
P = phosphorescence
S = singlet state
T = triplet state
IC = internal conversion
ISC = intersystem crossing

Perrin-Jablonski energy diagram (S_0, S_1 and S_2 transitions)




Potential energy
excited state singlet
triplet state
ground state singlet
absorption
fluorescence
phosphorescence
Inter-atomic distance

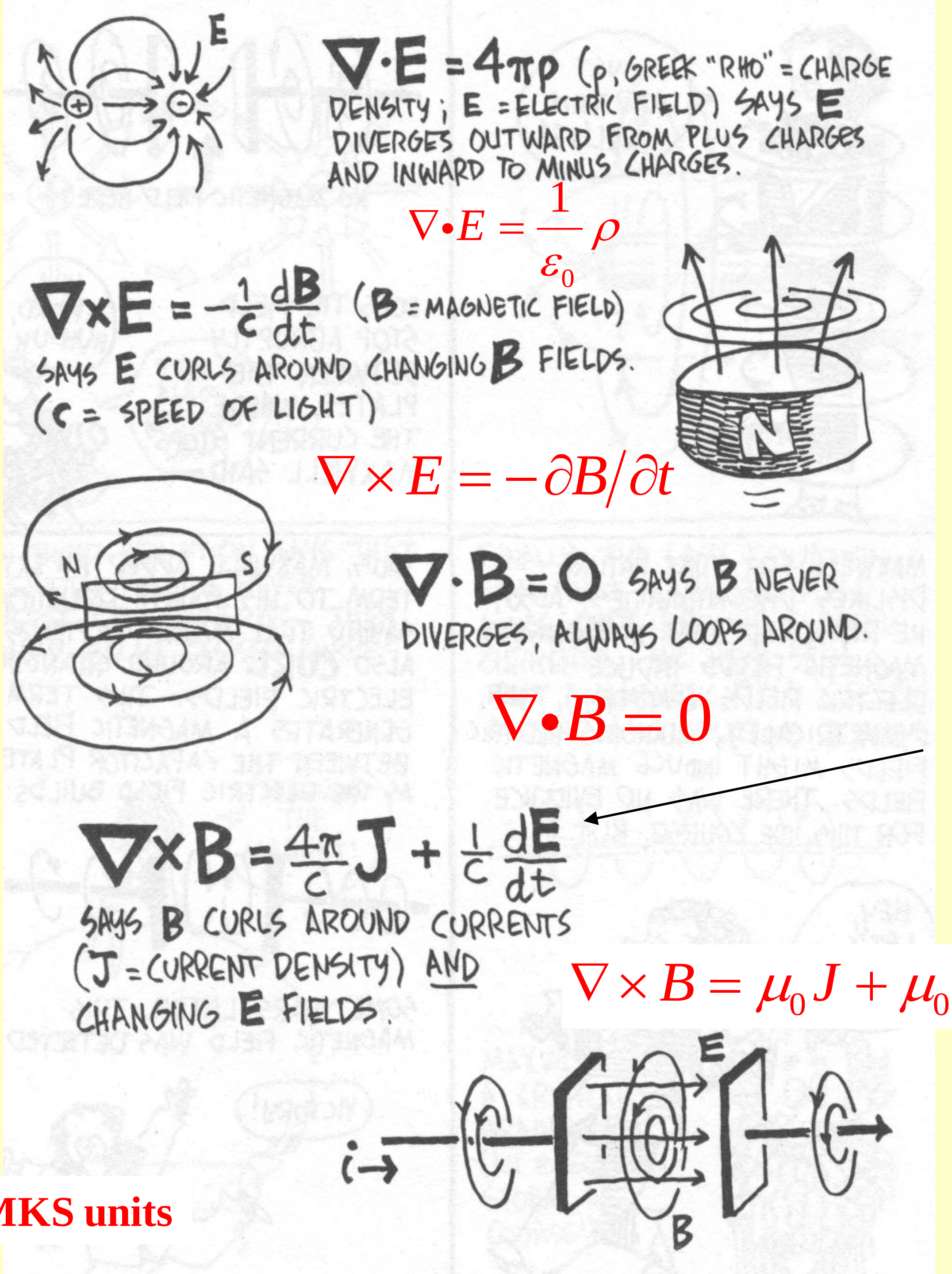
Why does a molecule emit radiation?

Classical point of view
Oscillating electric dipole (antenna)
Hertz Oscillator

Maxwell's Equations

Gauss Units

"$\nabla\times$" means to imagine a **"curling" component** of the field on which it acts. That is the field circles around in a certain direction at that point.



$\nabla \cdot E = 4\pi\rho$ (ρ, GREEK "RHO" = CHARGE DENSITY; E = ELECTRIC FIELD) SAYS E DIVERGES OUTWARD FROM PLUS CHARGES AND INWARD TO MINUS CHARGES.

$$\nabla \bullet E = \frac{1}{\varepsilon_0}\rho$$

$\nabla \times E = -\frac{1}{c}\frac{dB}{dt}$ (B = MAGNETIC FIELD) SAYS E CURLS AROUND CHANGING B FIELDS. (c = SPEED OF LIGHT)

$$\nabla \times E = -\partial B/\partial t$$

$\nabla \cdot B = 0$ SAYS B NEVER DIVERGES, ALWAYS LOOPS AROUND.

$$\nabla \bullet B = 0$$

$\nabla \times B = \frac{4\pi}{c}J + \frac{1}{c}\frac{dE}{dt}$ SAYS B CURLS AROUND CURRENTS (J = CURRENT DENSITY) AND CHANGING E FIELDS.

$$\nabla \times B = \mu_0 J + \mu_0\varepsilon_0\, \partial E/\partial t$$

You do NOT have to know these equations

This term was proven to be there several years later! This is a **VERY IMPORTANT TERM**, because it predicts **electromagnetic waves - or - LIGHT**!

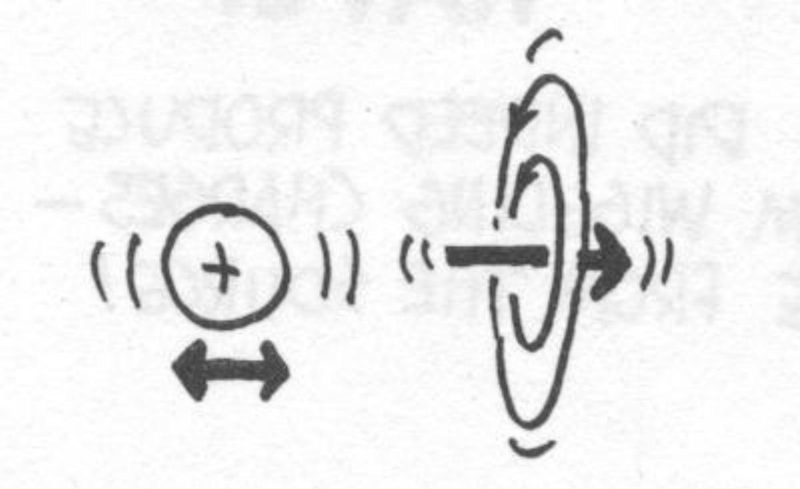
Red equations are MKS units

A classical "model" that is often used to understand why an atom emits light (and is correct for light scattering). It is useful, but only QM explains it correctly (see later).

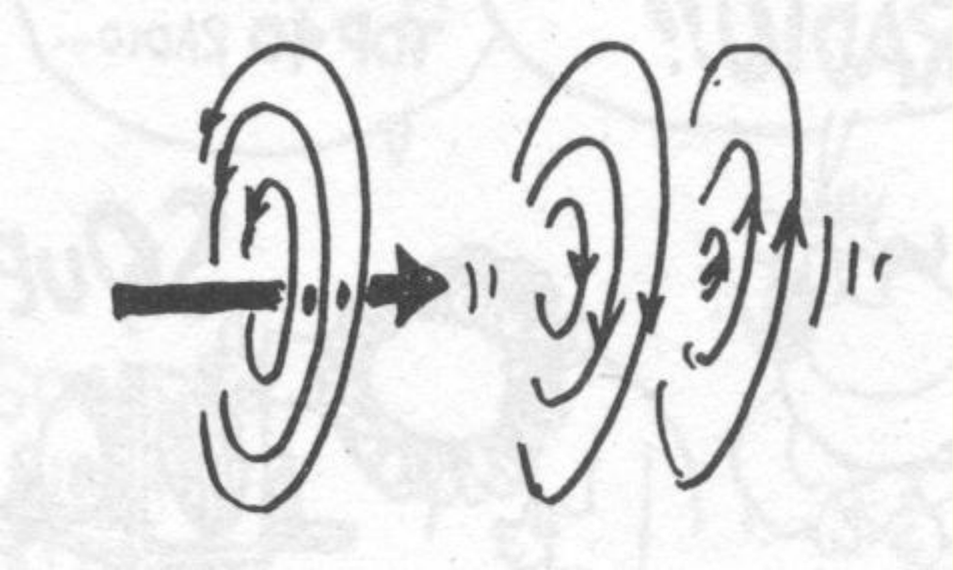
An accelerating (oscillating) charge emits radiation

Maxwell found this out; but never saw it proved

$$\nabla \times E = -\partial B/\partial t$$

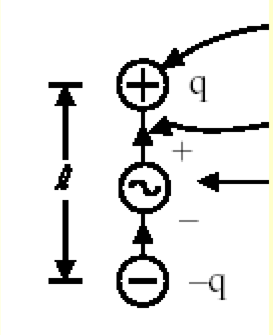


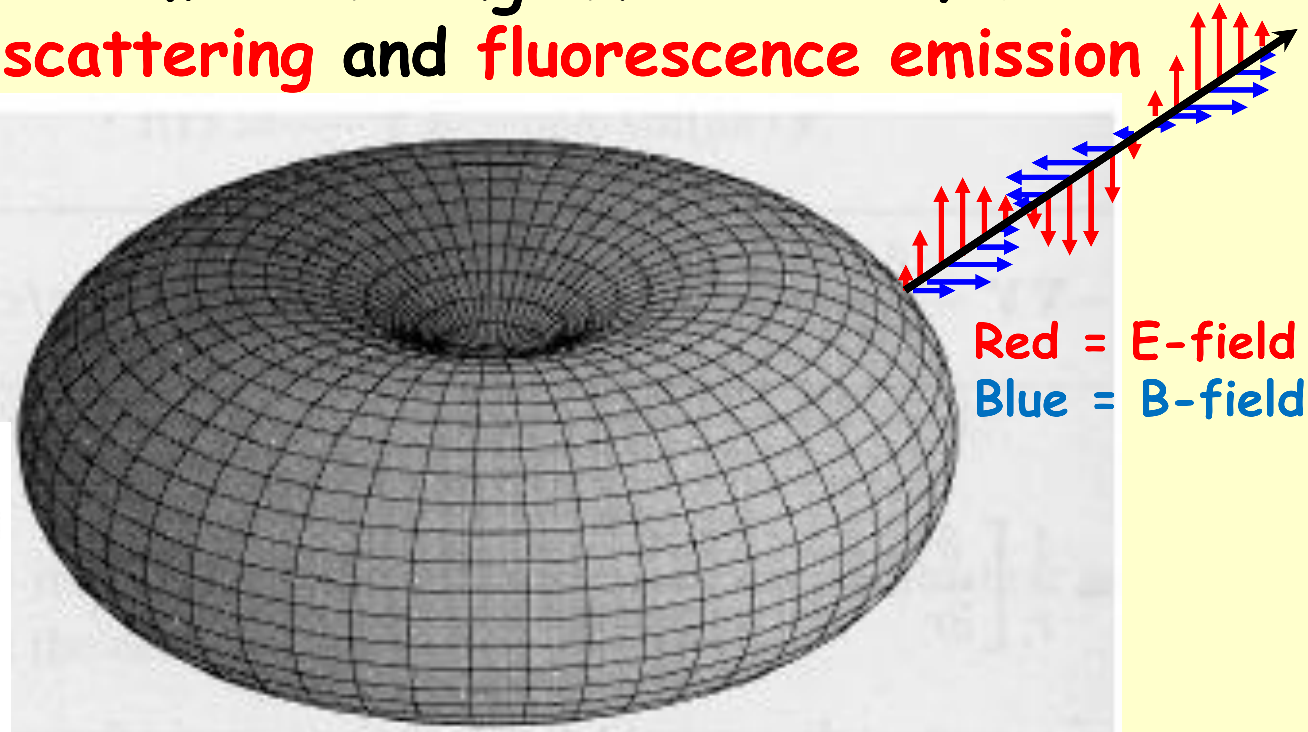
$$\nabla \times B = \mu_0 \varepsilon_0 \, \partial E/\partial t$$

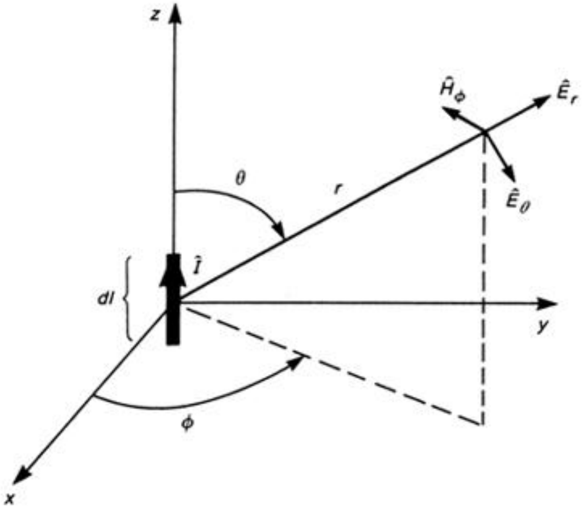


Hertz Dipole Radiation

This emission doughnut is valid for light scattering and fluorescence emission





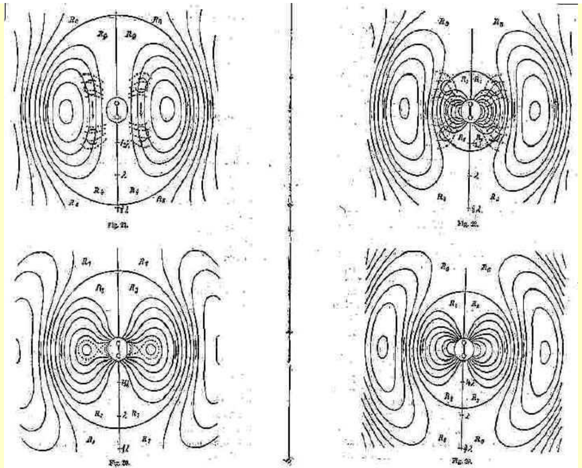


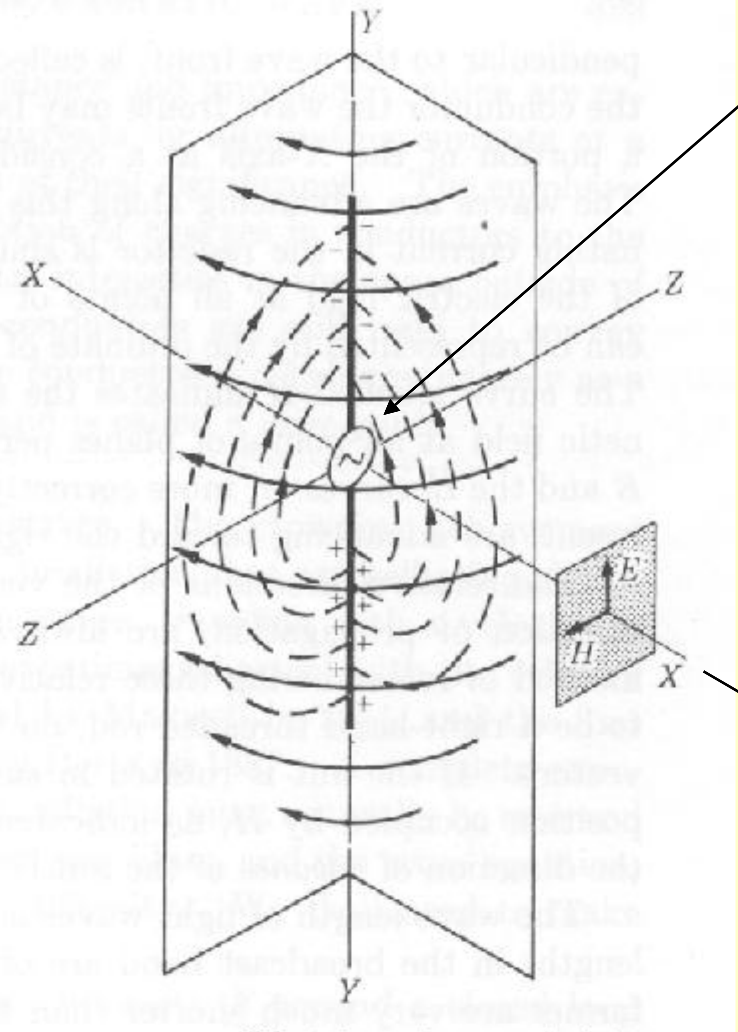
$$E_r = 60\beta^2 I\,dz\left[\frac{1}{(\beta r)^2} - \frac{j}{(\beta r)^3}\right]\cos\theta\, e^{-j\beta r}$$

$$E_\theta = j30\beta^2 I\,dz\left[\frac{1}{\beta r} - \frac{j}{(\beta r)^2} - \frac{1}{(\beta r)^3}\right]\sin\theta\, e^{-j\beta r}$$

$$H_\phi = j\frac{\beta^2}{4\pi} I\,dz\left[\frac{1}{\beta r} - \frac{j}{(\beta r)^2}\right]\sin\theta\, e^{-j\beta r}$$

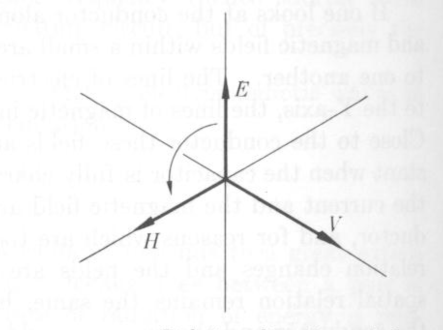
$$E_\phi = H_r = H_\theta = 0$$





Electric and magnetic fields around an oscillating dipole. Electric field, dotted lines; magnetic field, solid lines.

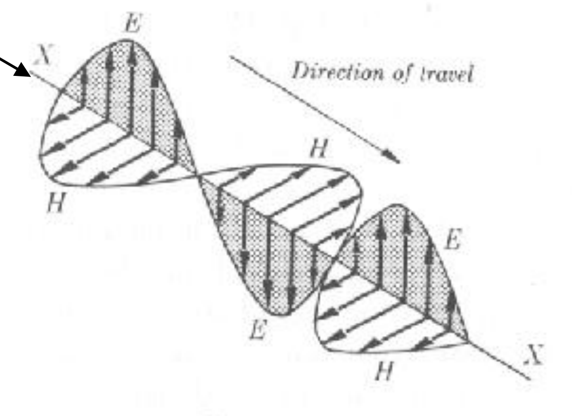
An oscillating (changing) electric charge will set up magnetic fields which will in turn cause electric fields, which will …



Relative directions of electric intensity E, magnetic intensity H, and velocity of propagation V in a plane polarized electromagnetic wave.

Solving the equations gives us a wave!

Far from the oscillating charge



Variations in electric and magnetic intensity in a traveling electromagnetic wave.

we

have

a

Light wave!

Free decay of oscillating real dipole
Emits radiative energy

A damped electric harmonic oscillator

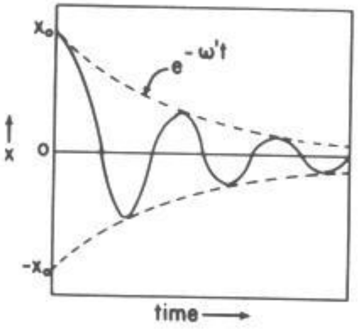


FIG. 15-4. Damped oscillations of an elastically bound electron. In the classical atom the damping would be much less rapid than is shown here.

$$\boxed{m_e \frac{d^2x}{dt^2} + (\eta+\mu)\frac{dx}{dt} + kx = 0}$$

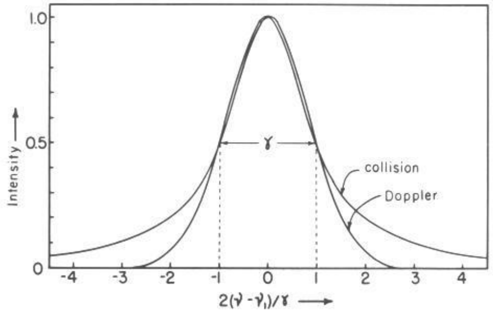


FIG. 15-7. Line shapes due to collision and radiation broadening and to Doppler broadening. Both curves have the same half-width, γ.

$$\Rightarrow \boxed{\frac{\gamma/2\pi}{(\nu-\nu_1)^2 + \left(\gamma/2\right)^2}}$$

Spectral distribution
Natural linewidth

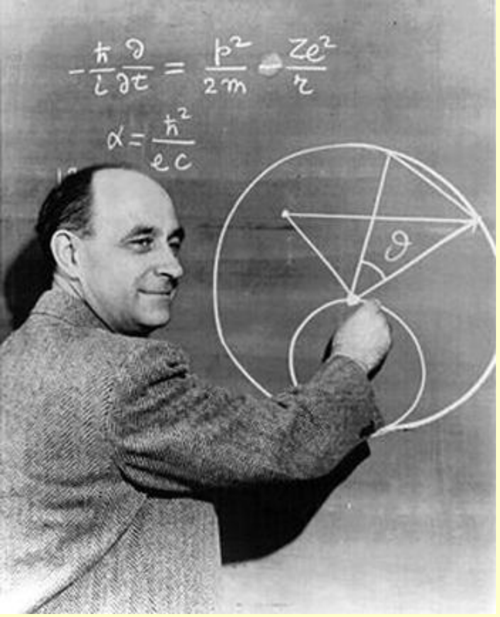
Actually gives the right fluorescence lifetime and spectral line width

$$m_e \frac{d^2x}{dt^2} + (\eta+\mu)\frac{dx}{dt} + kx = e\left|\vec{E}_0\right|\sin\omega t$$

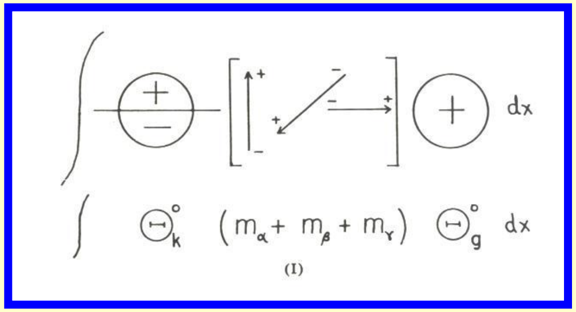
For absorption
Add forcing function

The correct theory for emission and absorption Is, of course, Quantum Mechanics

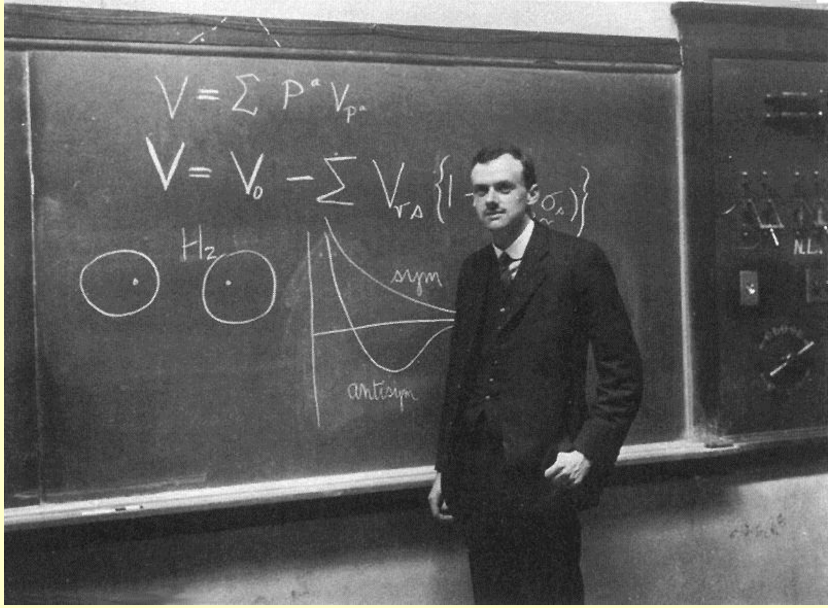
$$\boxed{\hat{H}\psi = -\hbar^2 / 2m\nabla^2\psi + V\psi = i\hbar\frac{\partial\psi}{\partial t}}$$



Transition moment



Transition Dipole
m=er



$$\lambda_{if} = \frac{2\pi}{\hbar}\left|M_{if}\right|^2 \rho_f$$

Fermi's Golden Rule

Transition probability

Matrix element for the interaction

Density of final states

OPPS - Dirac's Golden Rule

The "*attribution of Enrico Fermi's golden rule to Pauli is also miscast; it was Dirac who developed time-dependent perturbation theory, including this formula, to calculate radiative transitions with his other great invention, the quantized radiation field. More than 20 years later, Fermi, in his Chicago lectures, called the formula a golden rule, and many physicists, with their habitual disregard for history, have ever since attributed it to Fermi*"

KURT GOTTFRIED (*kg13@cornell.edu*) , *Cornell University , Ithaca, New York*

What do you do when there is no perturbation of the molecule in the excited state?

Stokes shift

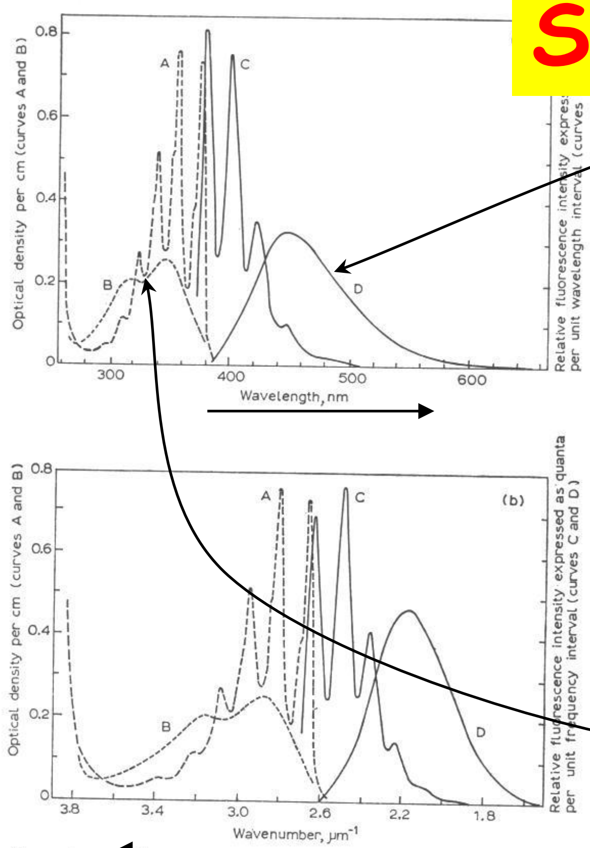


Fig. 2. Absorption and fluorescence emission spectra of anthracene and quinine bisulphate, illustrating the difference between plotting in units of (a) wavelength and energy and (b) wavenumber and quanta.

Curve A, absorption spectrum of anthracene in ethanol (17.2 μg per ml); curve B, absorption spectrum of quinine bisulphate in 0.1 N sulphuric acid (25 μg per ml); curve C, fluorescence emission spectrum of anthracene in ethanol (1.0 μg per ml); curve D, fluorescence emission spectrum of quinine bisulphate in 0.1 N sulphuric acid (1.0 μg per ml). Quartz prism spectrometers were used. The half-band width was 0.028 μm^{-1} (4.5 nm) at 2.5 μm^{-1} (400 nm) for fluorescence, and about 0.0005 μm^{-1} for absorption (from Parker and Rees[158]).

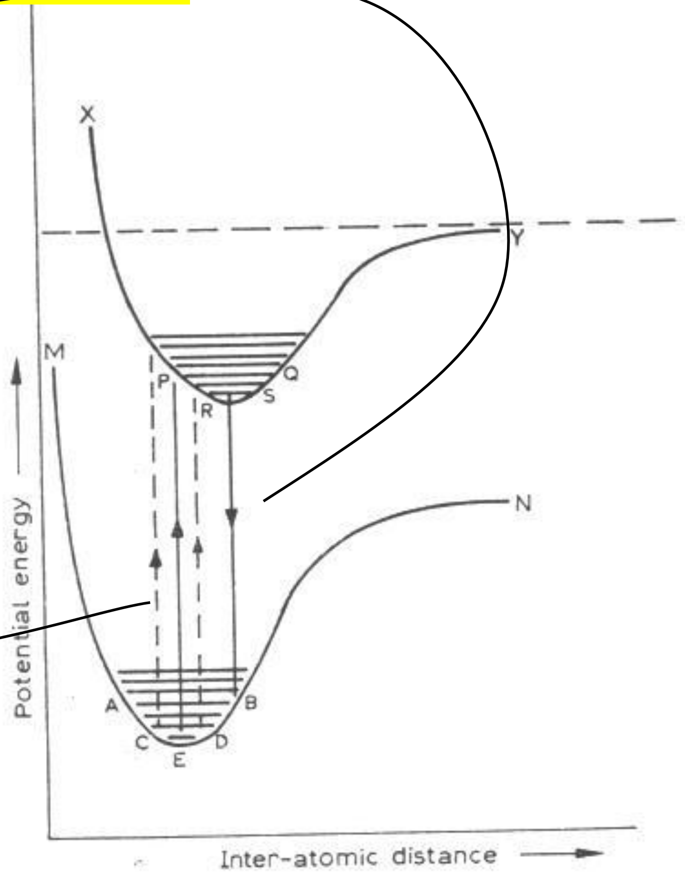


Fig. 3. The Franck–Condon principle

In the spectra we easily see the Stokes shift. We also see the 0-0 transition, which corresponds to the highest energy emitted by the excited molecule, and the lowest energy absorbed by the ground-state molecule.

Frank-Condon state transitions.
This is based on the fact that light is absorbed in 10^{-15} seconds, and the nuclei cannot move in this time. The electrons find a new spatial distribution in this time, but this is not the minimum energy configuration of the excited state.

Solvent Relaxation

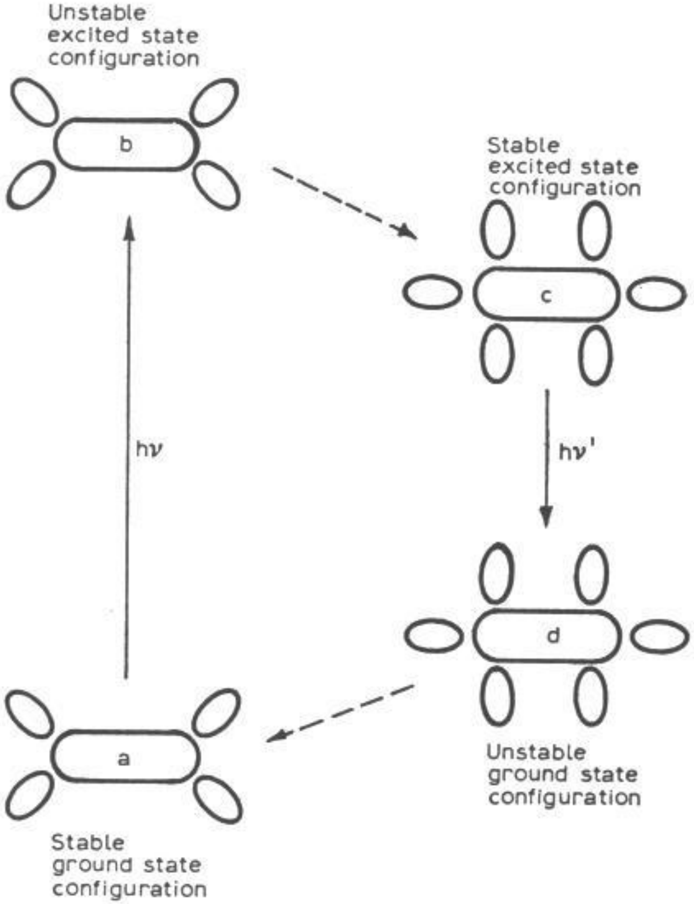


Fig. 4. Illustrating change of solvation after excitation or emission. The large and small ovals representing solute and solvating solvent molecules are purely diagrammatic and are intended to represent a higher degree of solvation in the stable configuration of the excited state.

Times of the different Transitions

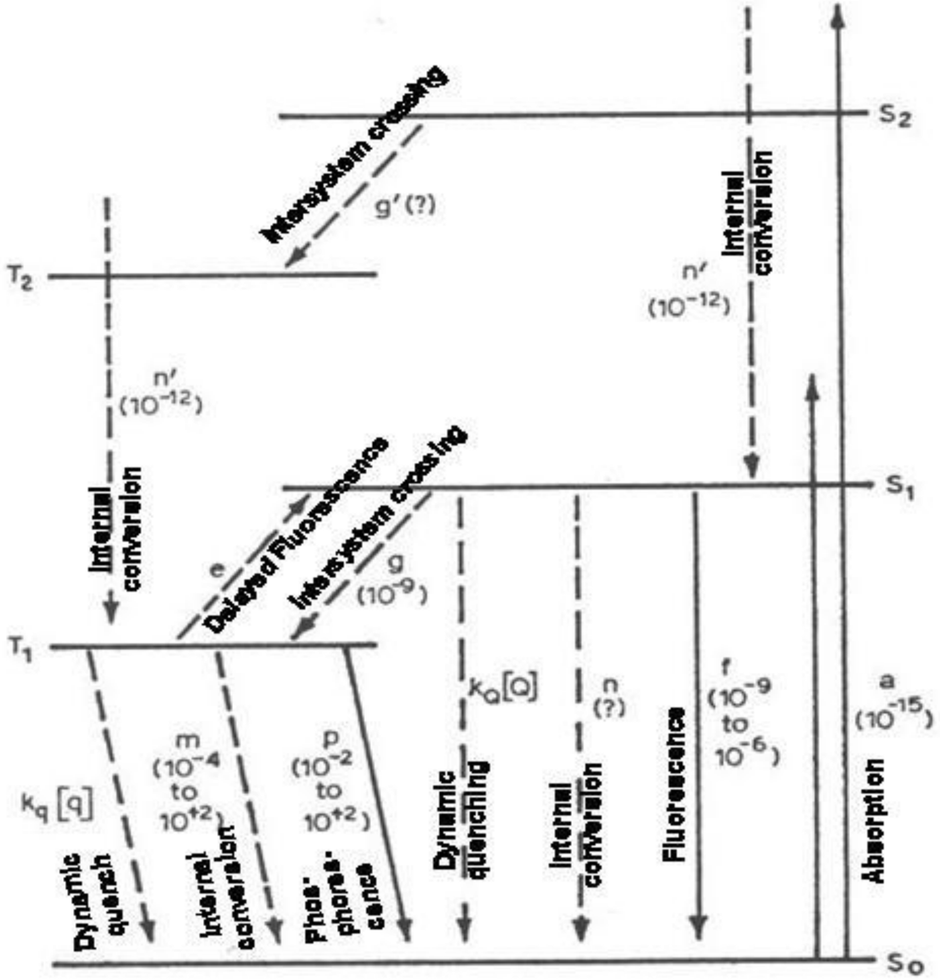


Fig. 23. Reciprocal rates of transitions (typical values).

Spins, triplets, singlets and all that

The transitions from S to T states, or from T to S states are formally forbidden (change in spin) and therefore they are usually slow (phosphorescence). They can be accelerated by spin-orbit coupling.

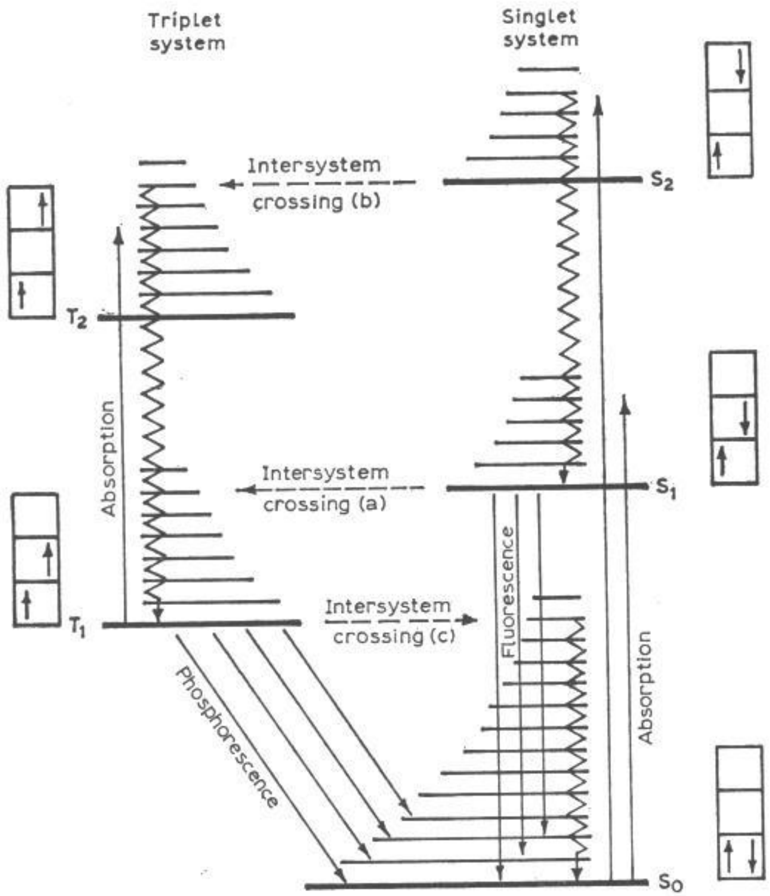


Fig. 12. Triplet and singlet energy levels and intersystem crossing.

Triplets are dangereous

The triplet state is very long lived, and therefore can react with the surrounding molecules. This is the electronic state that is most reactive, and is responsible for most of the destruction (photolysis and radical production). This is derived from the parallel spins.
The triplet state of a chromophore reacts with oxygen (which is a triplet in the ground state) to produce reactive oxygen species – ROS.

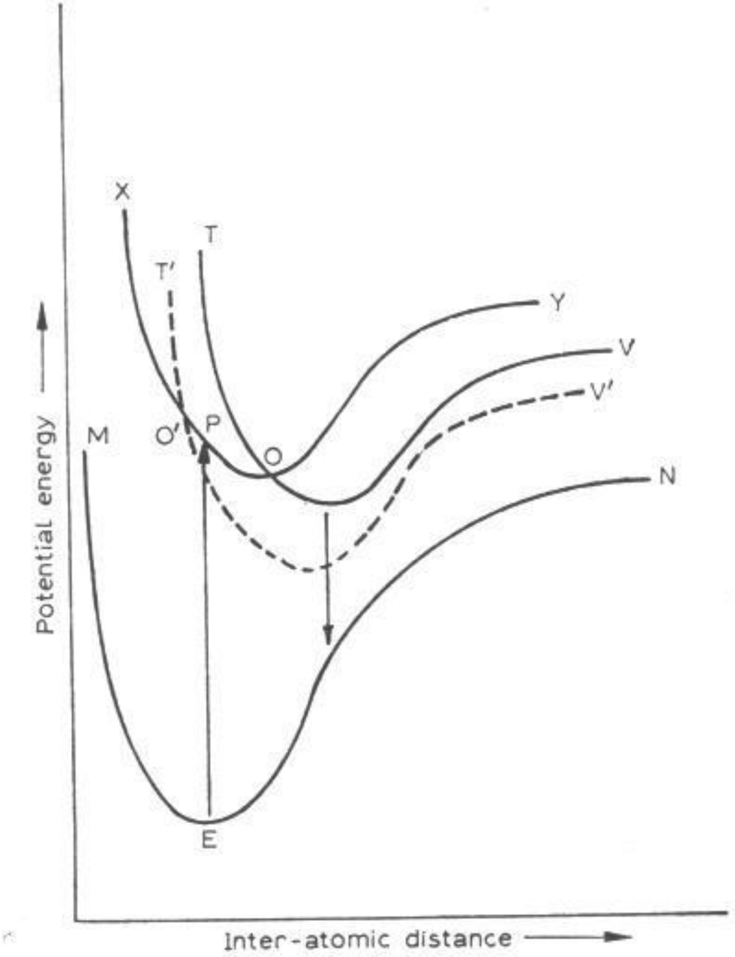


Fig. 13. Intersystem crossing.

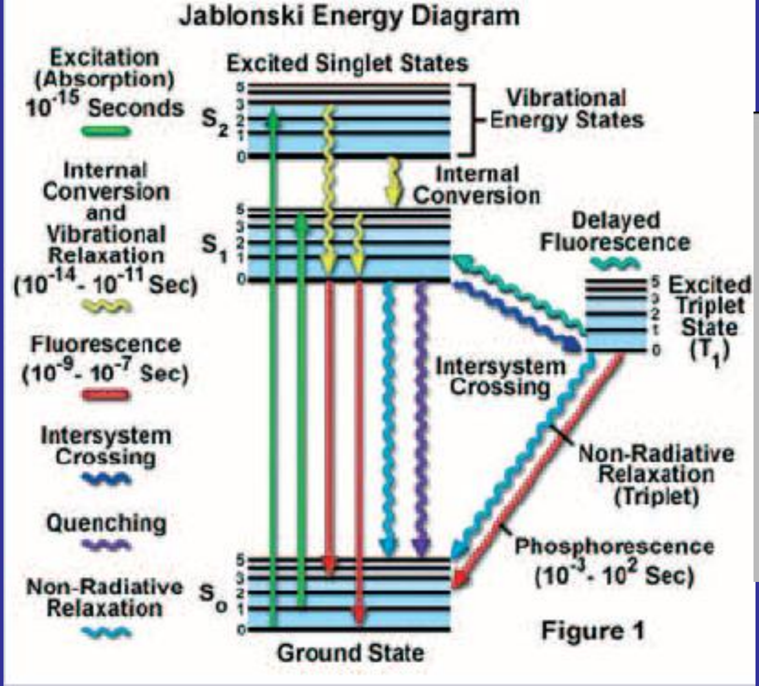
Light is polarized,
and this can be used to measure
molecular properties and is
important when measuring lifetimes
if the fluorophores can rotate
during the measurement

Light is polarized,
molecules interact with light *via* electric (transition) dipoles
the emitted light is polarized.

and polarization has consequences

A simple model for interpreting
the basics of
time dependent fluorescence anisotropy

Alexander Jablonski
(1898-1980)



Viele Jahrhunderte später beobachtete Nicolás Monardes *(1493-1588) am Extrakt von Lignum nephriticum – ein damals empfohlenes Arzneimittel bei Nierenbeschwerden – im Jahre 1565 erstmals Lumineszenzerscheinungen.*

Nicolás Monardes

1493 - 1588

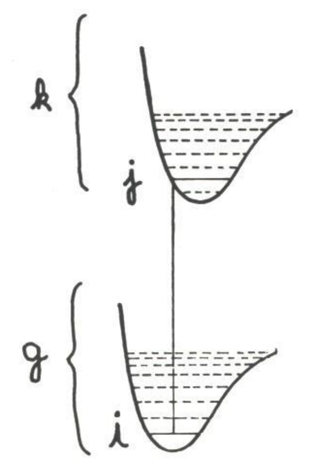
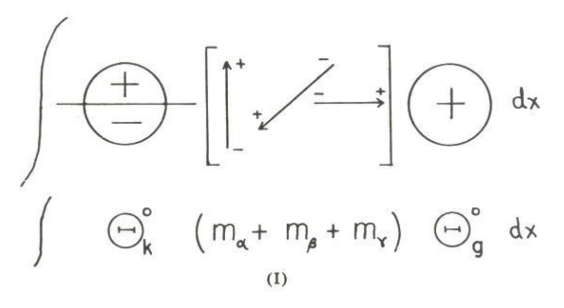


FIG. 1. Vibronic transition gi—kj

$$\vec{M}_{gi,kj} = \iint \Theta_k \phi_j^k \vec{m}(x) \Theta_g \phi_i^g \, dx\,dQ$$



Transition moment

Photoselection

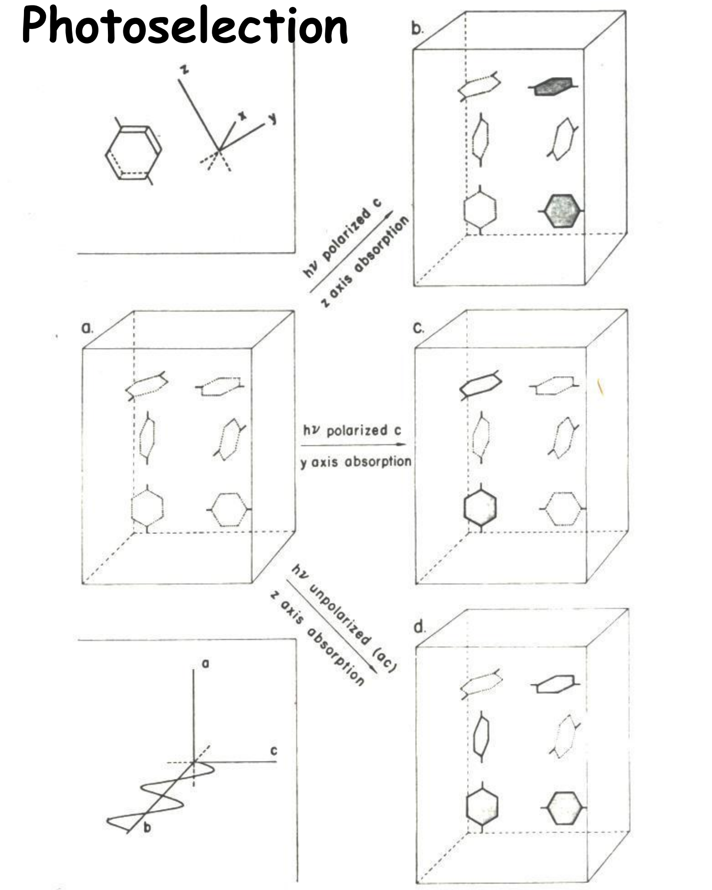
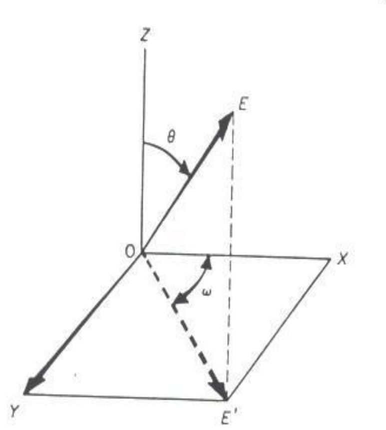
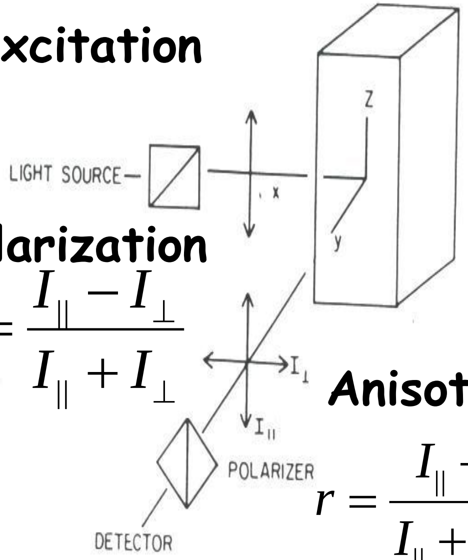


FIG. 2. Pictorial representation of photoselection. Upper left: the molecule fixed coordinate system. Lower left: the space fixed coordinate system. a: a representation of an isotropic sample. b, c, and d: the photoselected species (shaded) for various conditions of photoselection.



Rotational diffusion

Excitation



Polarization

$$p = \frac{I_\parallel - I_\perp}{I_\parallel + I_\perp}$$

Anisotropy

$$r = \frac{I_\parallel - I_\perp}{I_\parallel + 2 \bullet I_\perp}$$

Anisotropy decay Sphere

$$r = r_0 e^{-6kTt/f_r} = r_0 e^{-kTt/\eta V} = r_0 e^{-3t/\rho}$$

Rotational correlation coefficient

$$\rho = \frac{8\pi\eta r_s^3}{2kT} = \frac{3\eta V}{kT} \qquad V = \frac{4}{3}\pi r_s^3$$

Steady state anisotopy

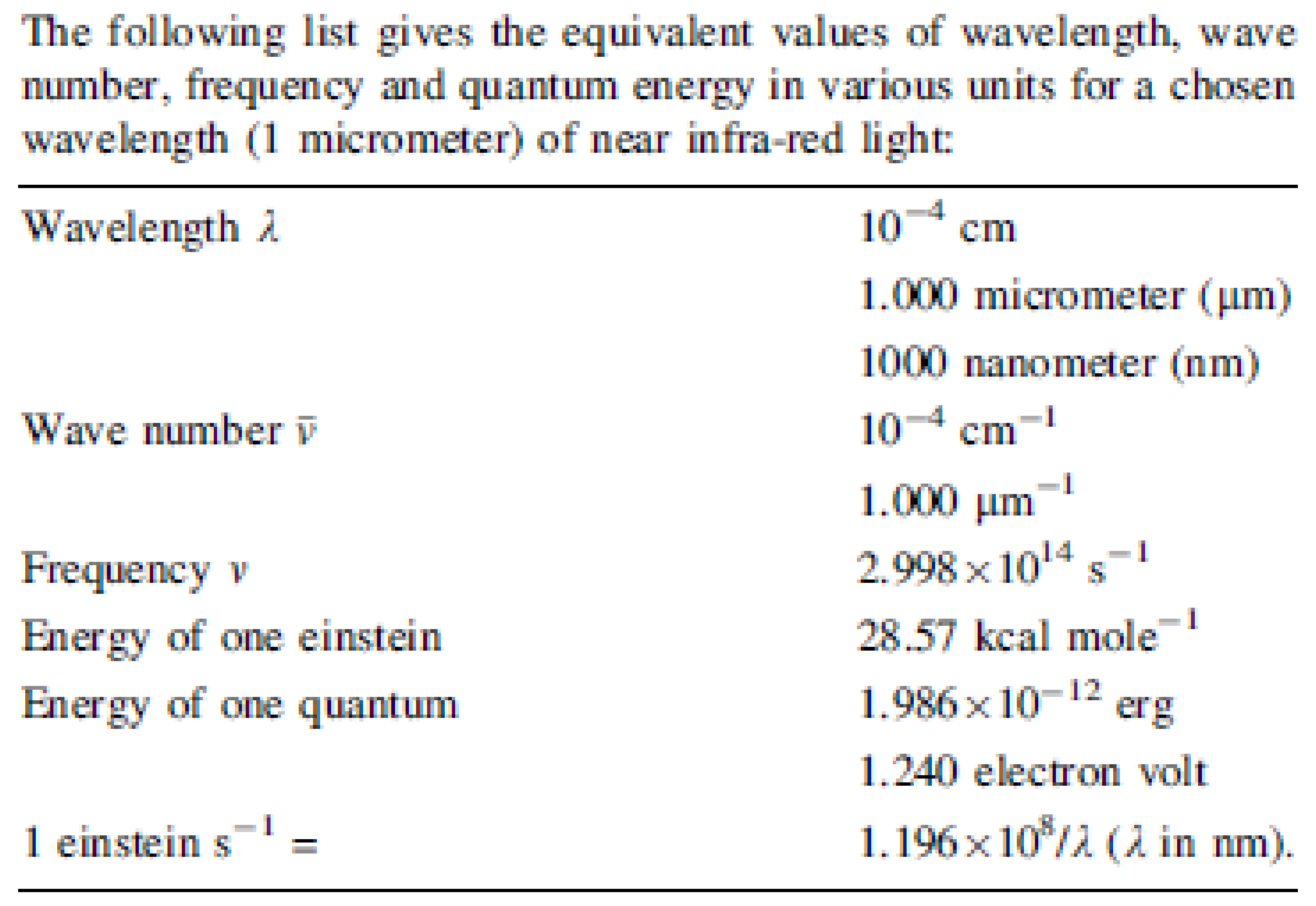
$$r = r_0 \left(1 + \frac{kT\tau}{\eta V}\right)^{-1}$$

This is a very important formula!

$E = h \cdot v$ $h = 6.6 * 10^{-34}$ Ws^2

The energy of a visible photon corresponding to light of a wavelength of 500nm (green) is about

$E = h(c/\lambda) = 4\ 10^{-19}$ Ws



The following list gives the equivalent values of wavelength, wave number, frequency and quantum energy in various units for a chosen wavelength (1 micrometer) of near infra-red light:

Wavelength λ	10^{-4} cm
	1.000 micrometer (μm)
	1000 nanometer (nm)
Wave number $\bar{\nu}$	10^{-4} cm^{-1}
	1.000 μm^{-1}
Frequency ν	2.998×10^{14} s^{-1}
Energy of one einstein	28.57 kcal mole^{-1}
Energy of one quantum	1.986×10^{-12} erg
	1.240 electron volt
1 einstein s^{-1} =	$1.196 \times 10^8/\lambda$ (λ in nm).

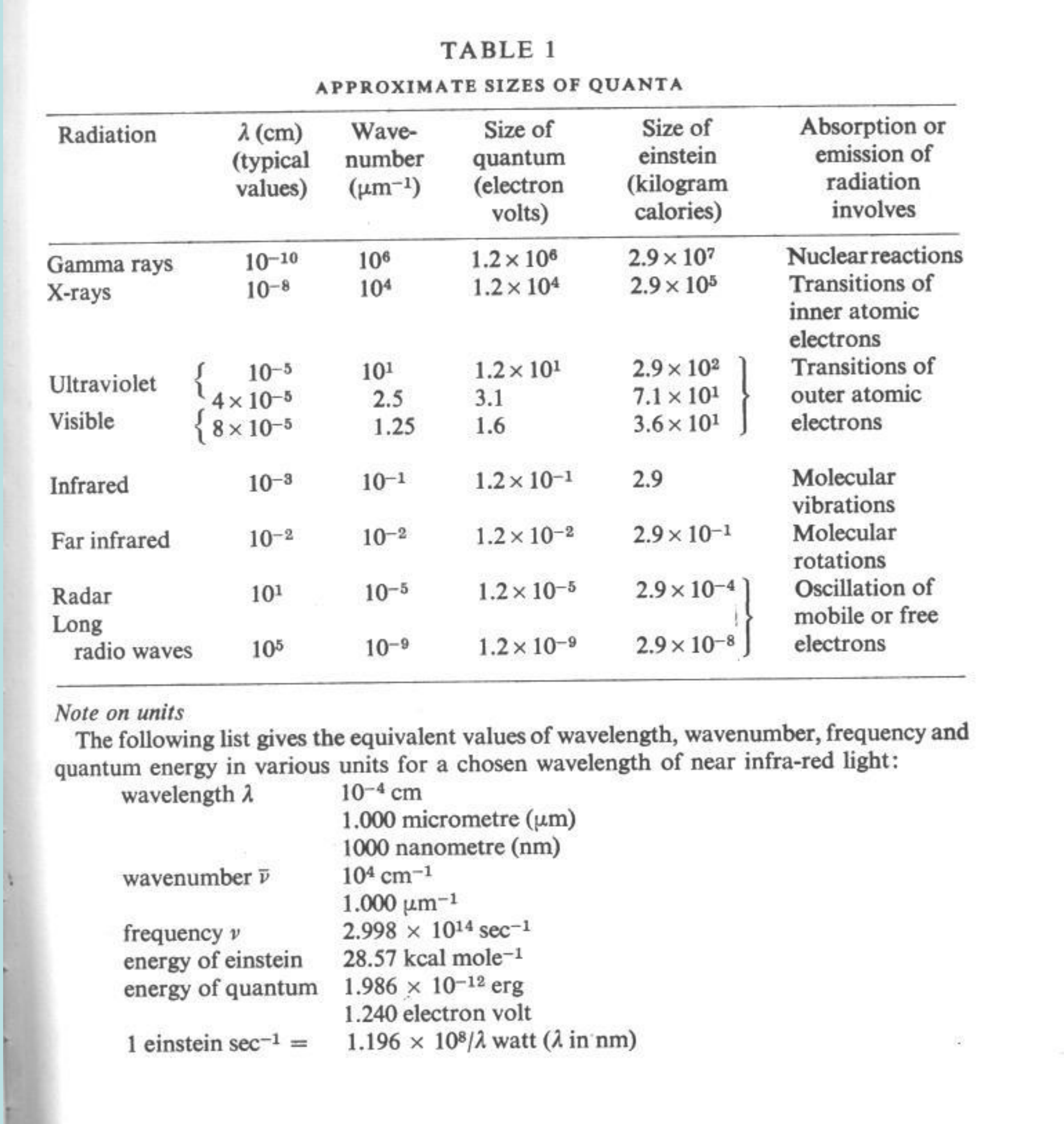


TABLE 1

APPROXIMATE SIZES OF QUANTA

Radiation	λ (cm) (typical values)	Wave-number (μm^{-1})	Size of quantum (electron volts)	Size of einstein (kilogram calories)	Absorption or emission of radiation involves
Gamma rays	10^{-10}	10^{6}	1.2×10^{6}	2.9×10^{7}	Nuclear reactions
X-rays	10^{-8}	10^{4}	1.2×10^{4}	2.9×10^{5}	Transitions of inner atomic electrons
Ultraviolet	10^{-5}	10^{1}	1.2×10^{1}	2.9×10^{2}	Transitions of outer atomic electrons
	4×10^{-5}	2.5	3.1	7.1×10^{1}	
Visible	8×10^{-5}	1.25	1.6	3.6×10^{1}	
Infrared	10^{-3}	10^{-1}	1.2×10^{-1}	2.9	Molecular vibrations
Far infrared	10^{-2}	10^{-2}	1.2×10^{-2}	2.9×10^{-1}	Molecular rotations
Radar	10^{1}	10^{-5}	1.2×10^{-5}	2.9×10^{-4}	Oscillation of mobile or free electrons
Long radio waves	10^{5}	10^{-9}	1.2×10^{-9}	2.9×10^{-8}	

Note on units

The following list gives the equivalent values of wavelength, wavenumber, frequency and quantum energy in various units for a chosen wavelength of near infra-red light:

wavelength λ	10^{-4} cm
	1.000 micrometre (μm)
	1000 nanometre (nm)
wavenumber $\bar{\nu}$	10^{4} cm^{-1}
	1.000 μm^{-1}
frequency ν	2.998×10^{14} sec^{-1}
energy of einstein	28.57 kcal mole^{-1}
energy of quantum	1.986×10^{-12} erg
	1.240 electron volt
1 einstein sec^{-1} =	$1.196\times10^{8}/\lambda$ watt (λ in nm)

OK!
I understand everything now

I think