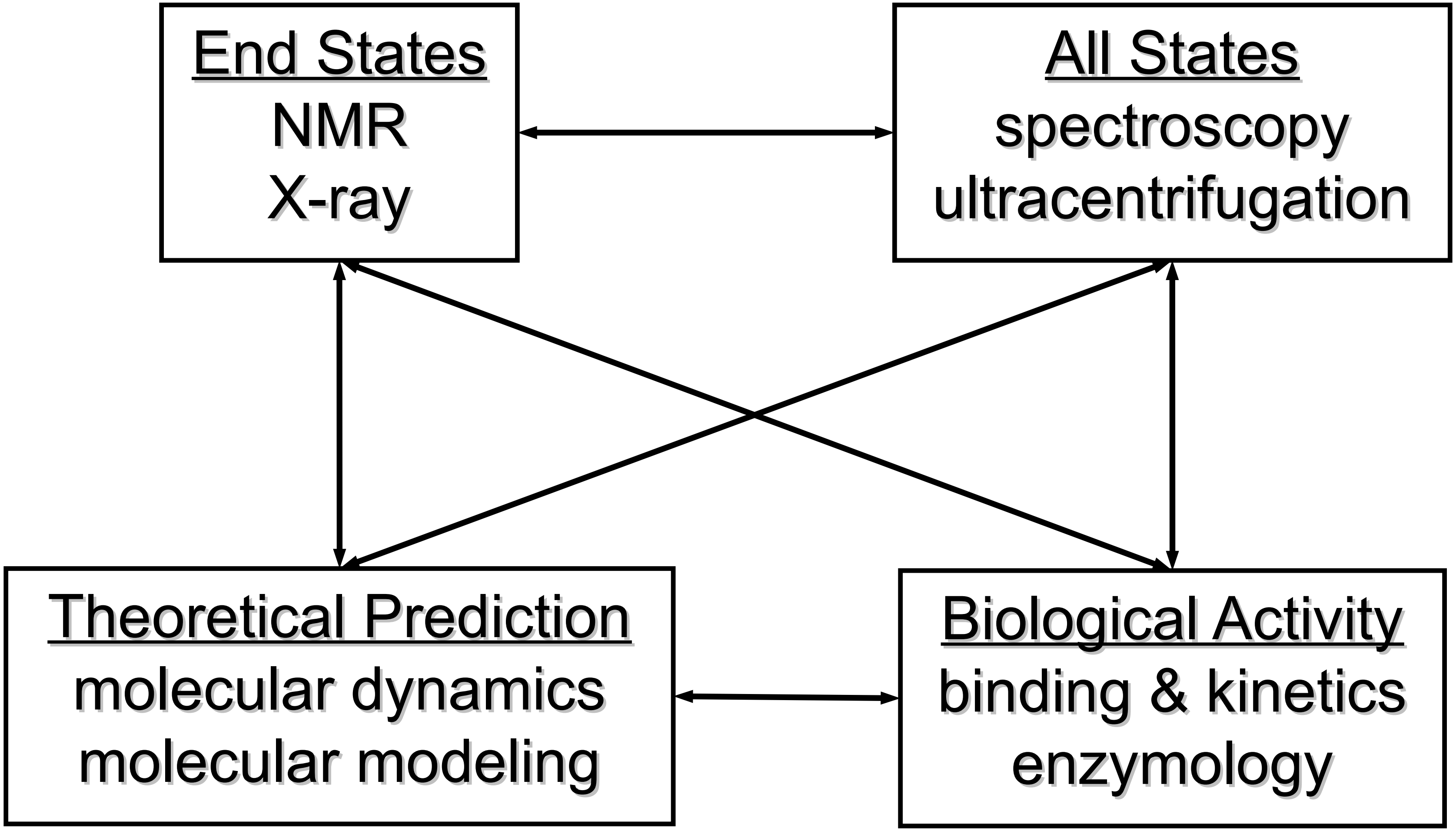
Spectroscopy

STRUCTURE – MOTION – FUNCTION



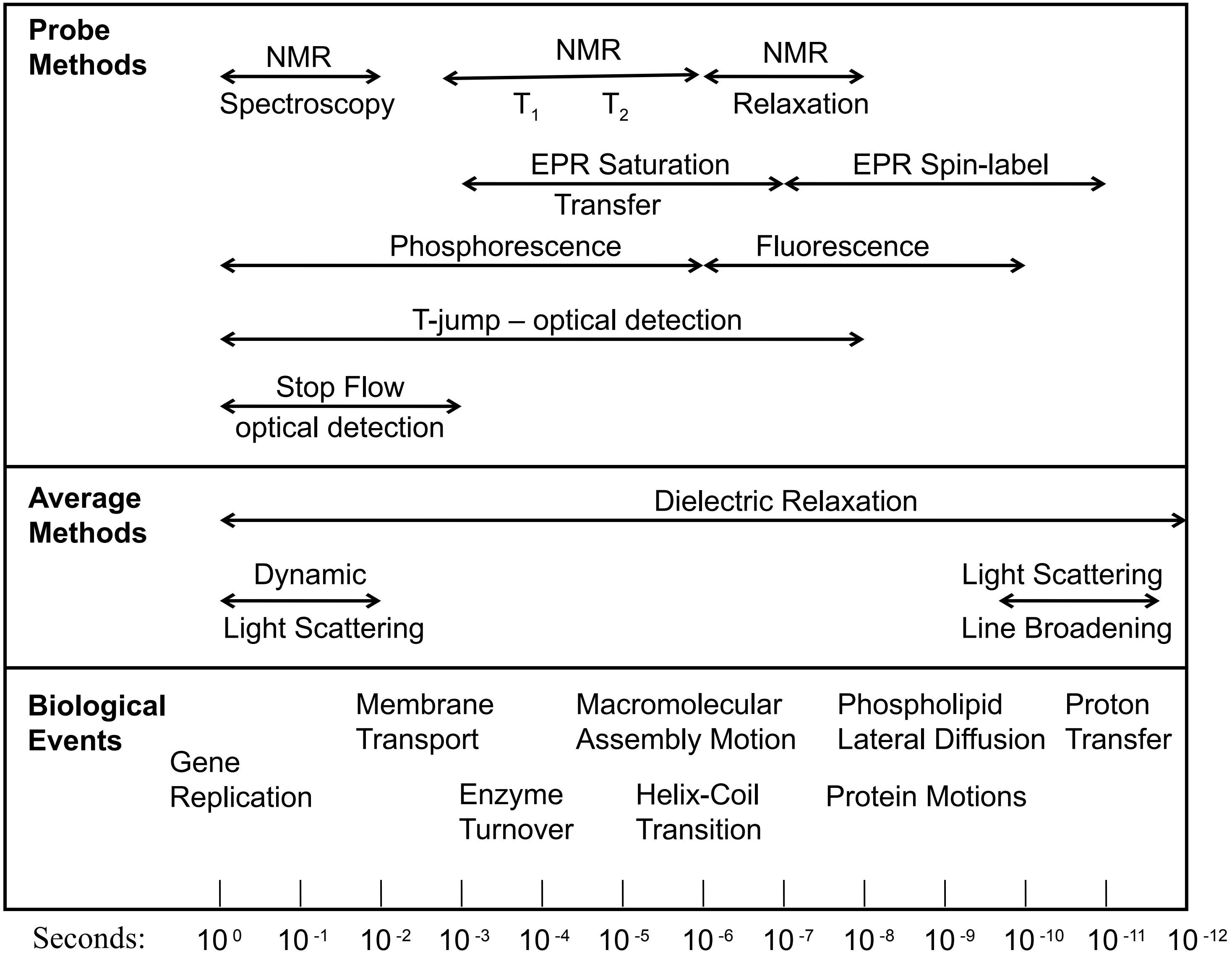
End States
NMR
X-ray

All States
spectroscopy
ultracentrifugation

Theoretical Prediction
molecular dynamics
molecular modeling

Biological Activity
binding & kinetics
enzymology

Spectroscopy



Probe Methods

NMR Spectroscopy

NMR T_1 T_2

NMR Relaxation

EPR Saturation Transfer

EPR Spin-label

Phosphorescence

Fluorescence

T-jump – optical detection

Stop Flow optical detection

Average Methods

Dielectric Relaxation

Dynamic Light Scattering

Light Scattering Line Broadening

Biological Events

Gene Replication

Membrane Transport

Enzyme Turnover

Macromolecular Assembly Motion

Helix-Coil Transition

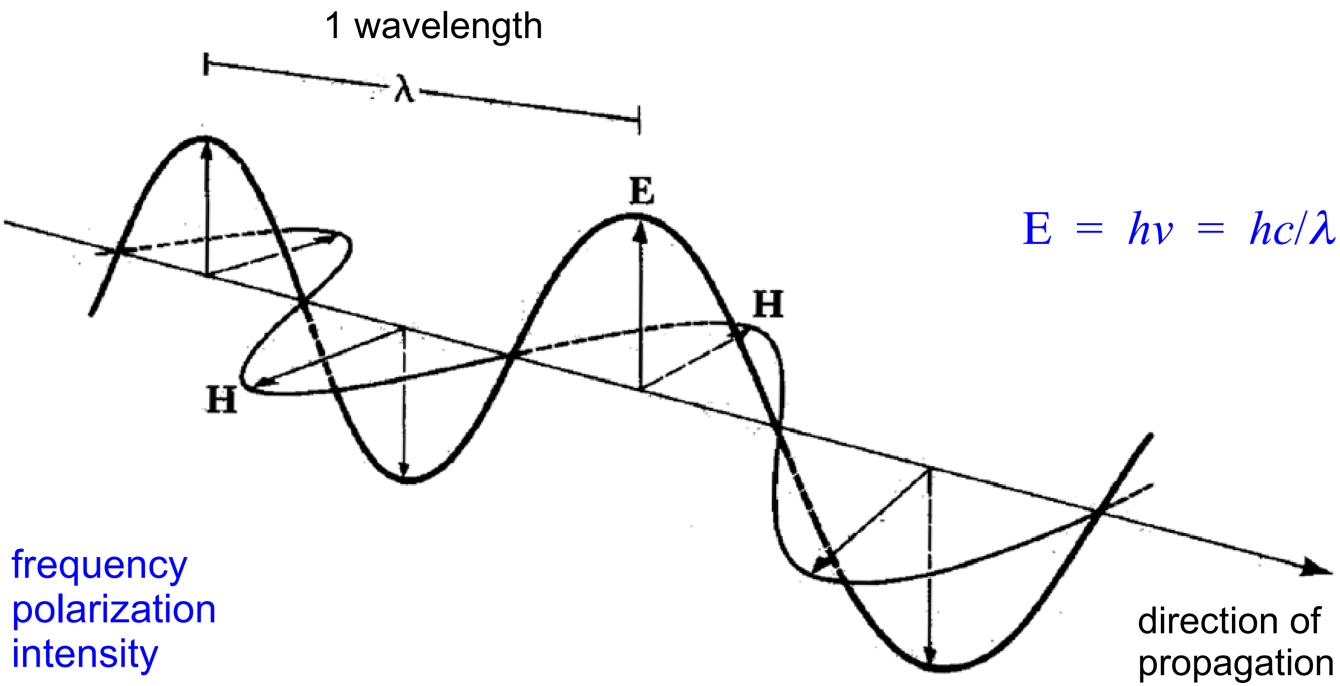
Phospholipid Lateral Diffusion

Protein Motions

Proton Transfer

Seconds: 10^{0} 10^{-1} 10^{-2} 10^{-3} 10^{-4} 10^{-5} 10^{-6} 10^{-7} 10^{-8} 10^{-9} 10^{-10} 10^{-11} 10^{-12}

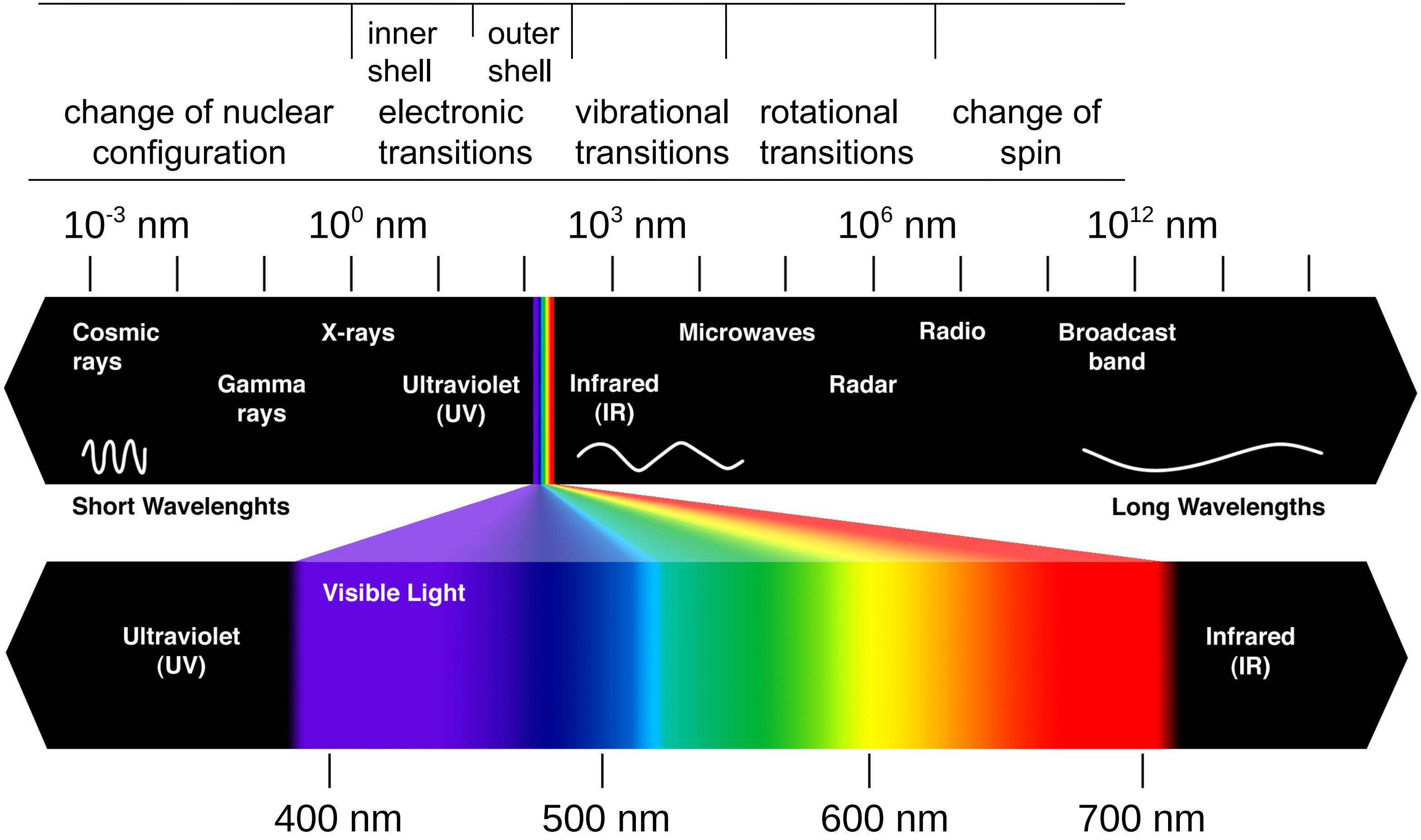
Electromagnetic Radiation



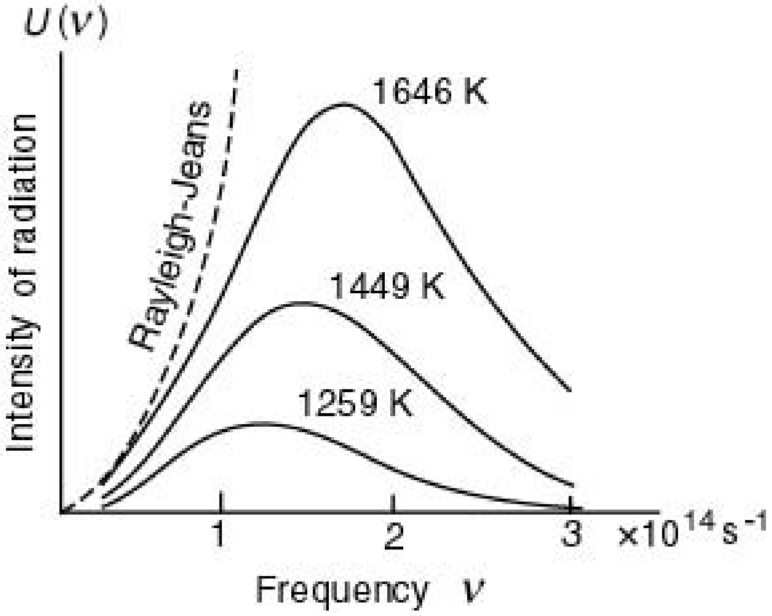
$$E = h\nu = hc/\lambda$$

- frequency
- polarization
- intensity

Spectroscopy



Spectroscopy



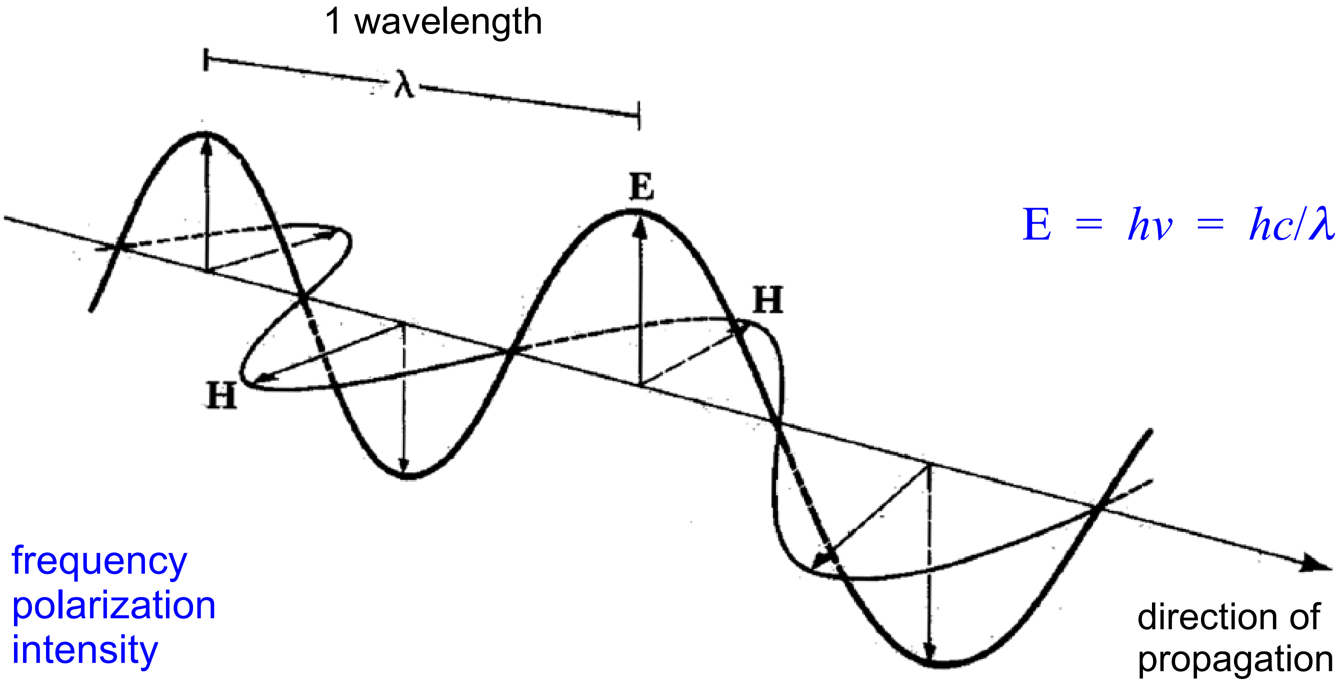
Planck's Function

$$B_\lambda(T) = \frac{2hc^2}{\lambda^5\left(e^{hc/k_B \lambda T} - 1\right)}$$

- Radiation formula derived by Max Planck in 1901
- Gives the spectral radiance emitted by a blackbody at temperature T (K) *in the direction normal to the surface*
- Units?
- *c*: speed of light (2.998×10^{8} m s^{-1})
- *h*: Planck's constant (6.626×10^{-34} J s)
- k_B: Boltzmann's constant (1.381×10^{-23} J K^{-1}) – relates energy at the particle level to temperature
- Note that this form of the Planck function uses wavelength (λ)

$$E = h\nu = hc/\lambda$$

Electromagnetic Radiation



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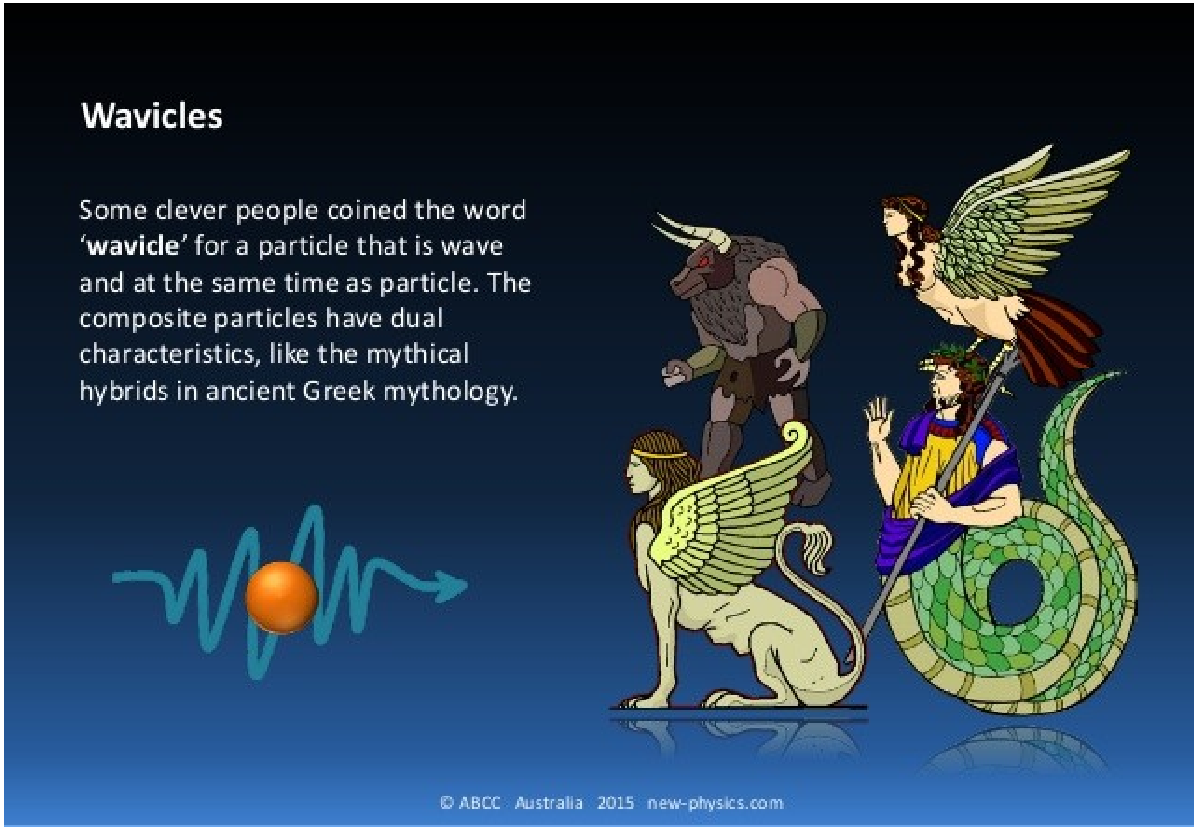
Spectroscopy



Wavicles

Some clever people coined the word '**wavicle**' for a particle that is wave and at the same time as particle. The composite particles have dual characteristics, like the mythical hybrids in ancient Greek mythology.

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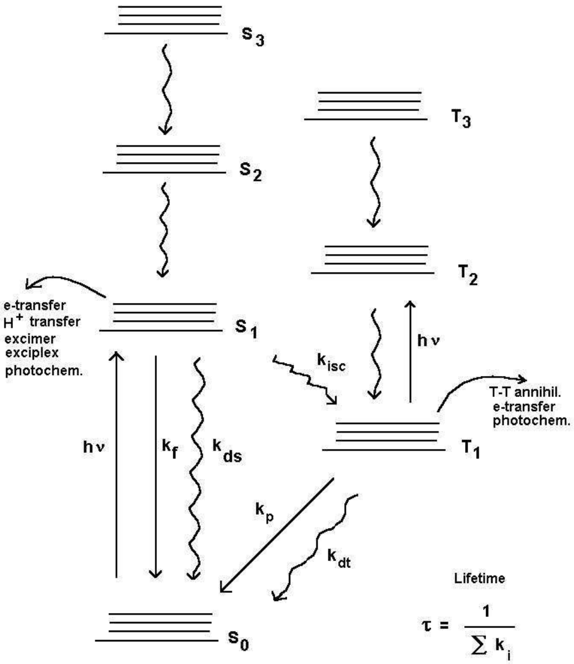
Spectroscopy

Aleksander Jabłoński



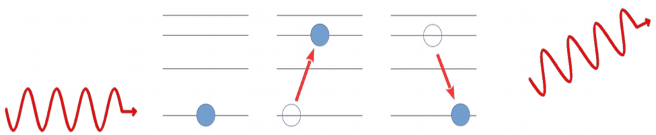
1934 <u>habilitation</u>

“On the influence of intermolecular interactions on the absorption and emission of light”

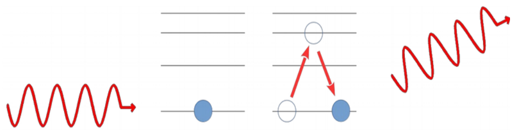
Lakowicz, Principles Fluorescence Spectroscopy 3rd Ed., 2006

Spectroscopy

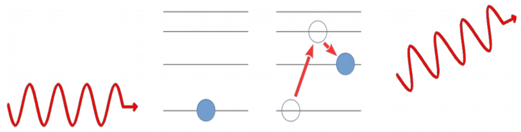
Photon interactions with an atom



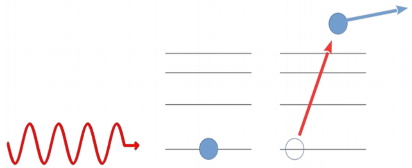
Resonance absorption.
Absorbs photon, later decays, incident and emitted photons uncorrelated.



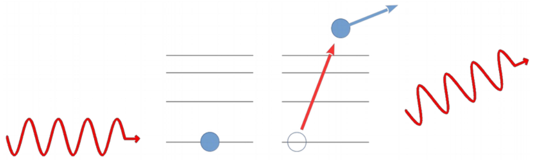
Rayleigh scattering, elastic, correlated.



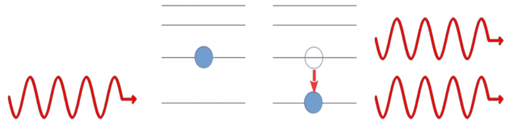
Raman scattering, inelastic, correlated.
Energy of emitted photon can be less or more than incident (latter would require a modification to the Jabłonski diagram).



Photoelectric effect.



Compton effect, inelastic.



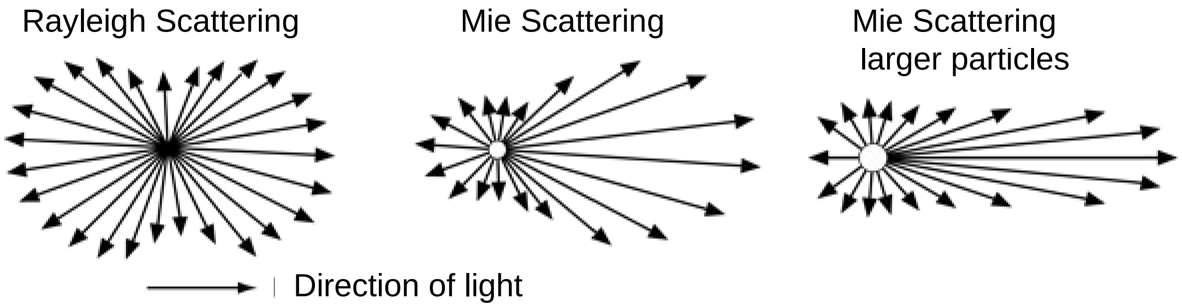
Stimulated emission, incident and emitted photons have the same energy, coherent.

Spectroscopy

Size of scatterer and wavelength of incident light

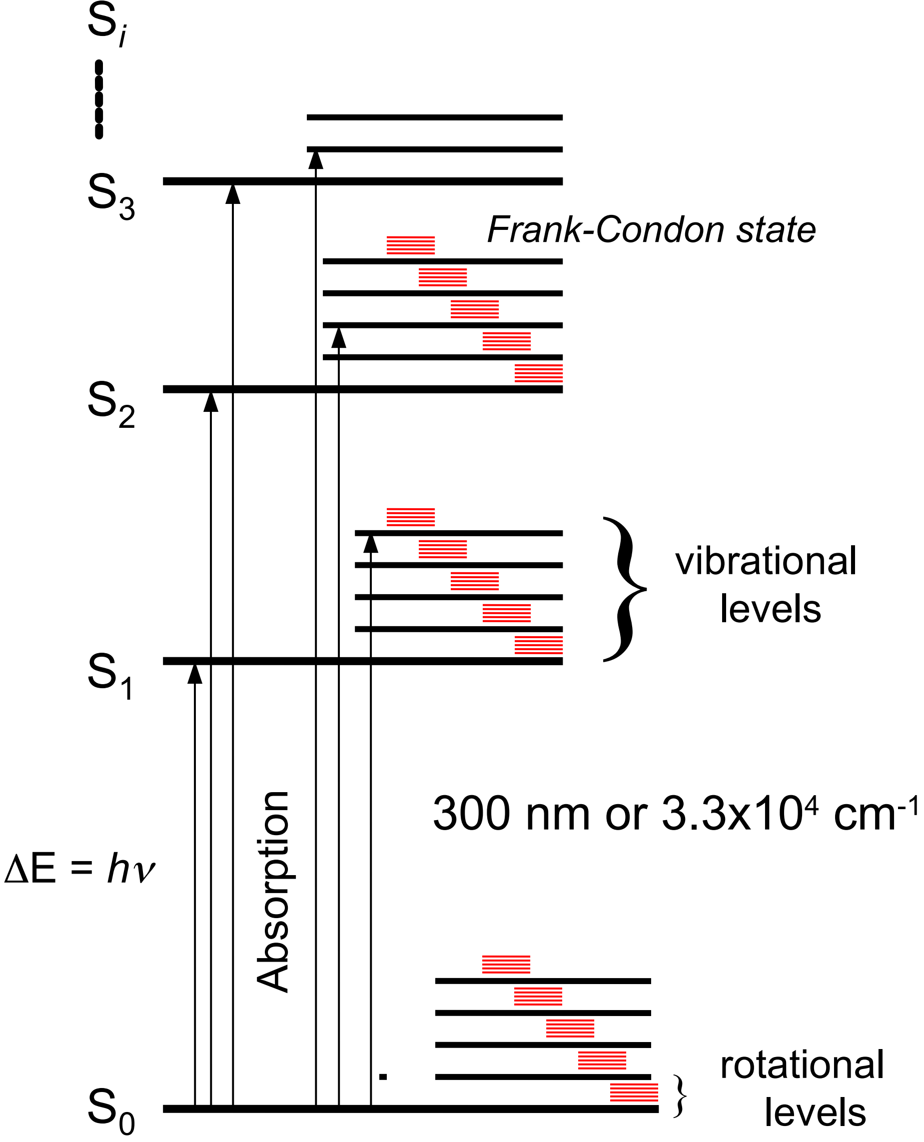
If the wavelength of the incident light approaches the size of the scatterer, Rayleigh scattering changes to Mie scattering.

The Rayleigh scattering model breaks down when the size of the scatterer becomes larger than around 10% of the wavelength of the incident light.



Spectroscopy

Jabłonski Diagram



Time Scales

Abs.: ~ 10^{-15} s

Spectroscopy

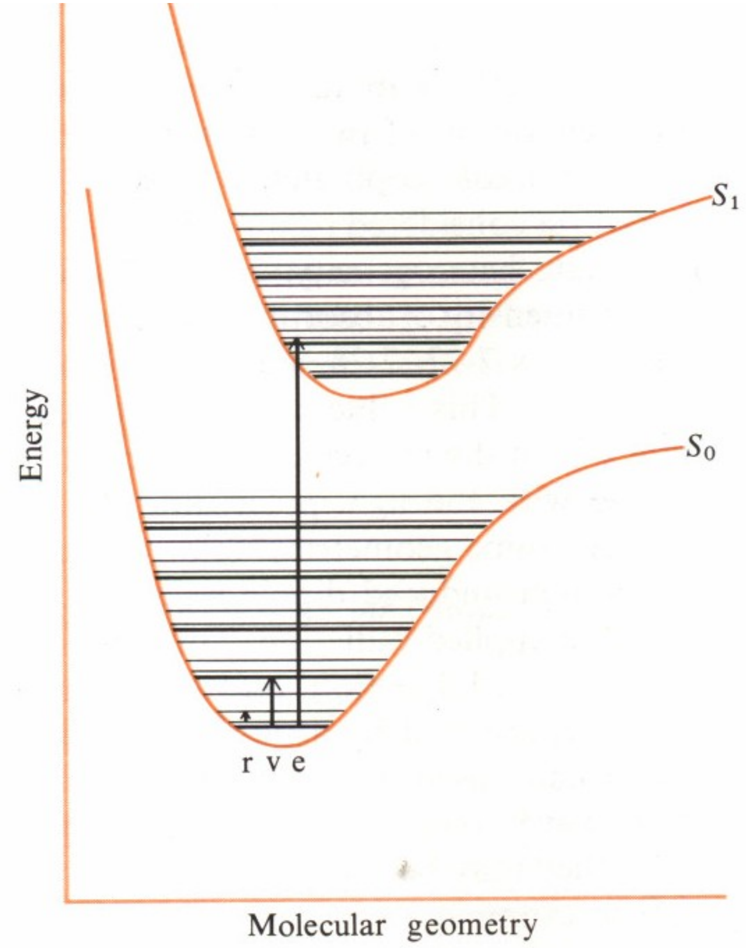
- Energy levels of a small molecule. Transitions occur between electronic states (e), vibrational (v) and rotational (r) levels. There can be many energy levels making absorbance bands broad.

- Energy spacing between electronic states is around 80 kcal/mol → higher than room temperature, only possible by absorbing light

- Energy spacing between vibrational leves is about 10 kcal/mole, higher than thermal energies, so only the ground states are normally populated

- Energy spacing between rotational levels is about 1 kcal/mol, sufficiently small for multiple levels to be populated at room temperature.

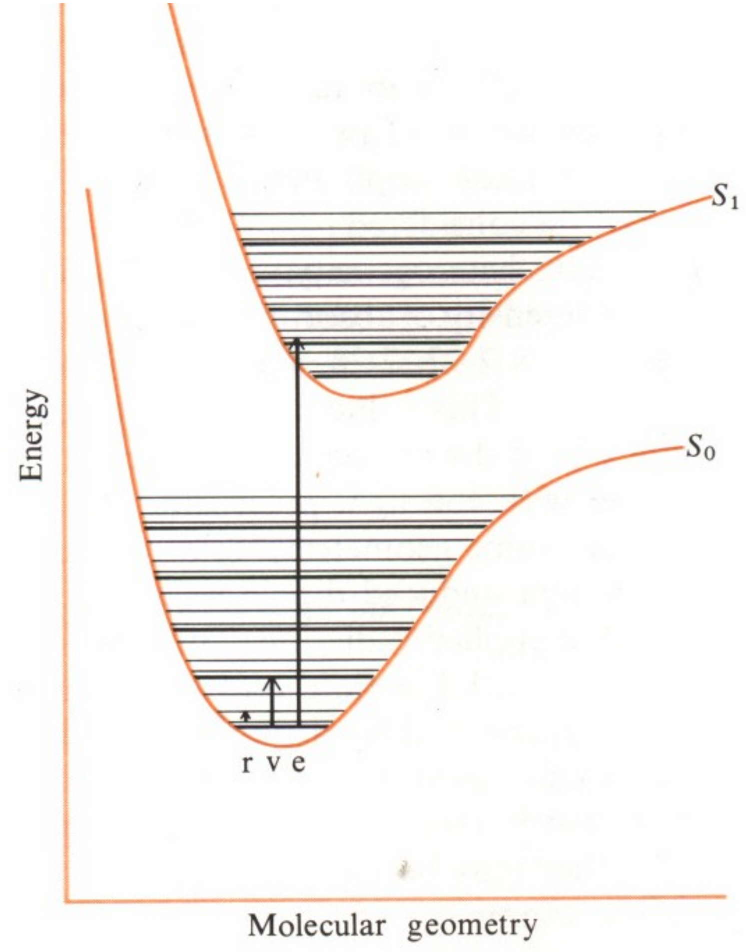
- The energy spacing between <u>vibrational</u> and <u>electronic</u> states is large that molecules are practically in their ground states at room temperature.



Spectroscopy

- The energy spacing between <u>vibrational</u> and <u>electronic</u> states is large that molecules are practically in their ground states at room temperature.

- The Franck-Condon principle states that electronic transitions that involve different vibrational levels have the highest probability of occurring when the vibrational levels overlap in the momentum and nuclear positions, i.e., no displacement of nuclei.



Energies required for various transitions:

- Electronic transitions: UV/visible light

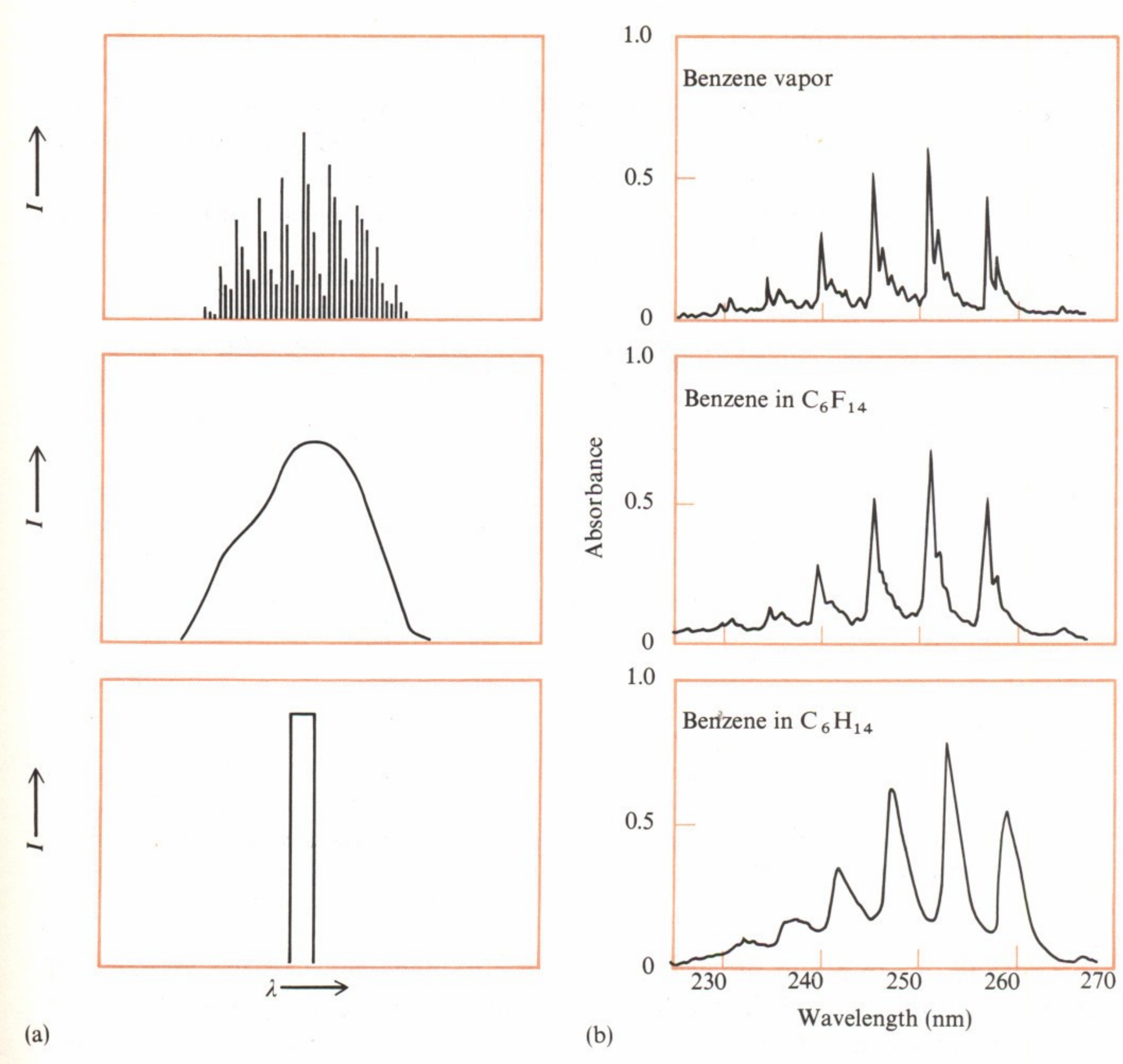
- Vibrational transitions: Infrared light

- Rotational transitions: microwave radiation, not suitable for absorbance spectroscopy, they will be considered with nuclear magnetic resonance.

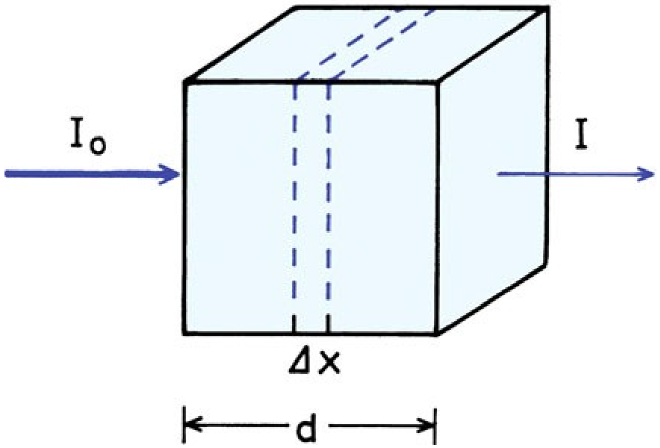
Spectroscopy

Absorption spectrum of a small molecule (a) and benzene (b) in the gas phase (top), the solution phase (center) and the theoretically calculated spectrum (bottom).

Observed spectra are generally broad envelopes of many closely spaced sharp spectral transitions. Further broadening of the spectrum is caused by loca solvent heterogeneity, Doppler shifts and other effects.



Beer-Lambert Law (of absorbance)



absorption cross-section:

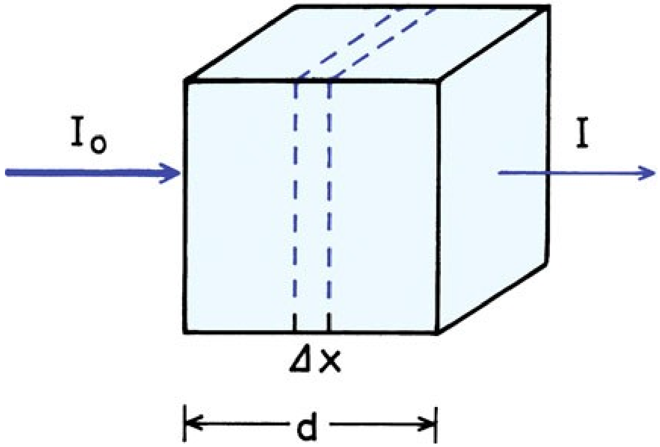
$k = p\,\sigma$; [cm^2]

incoming intensity, I_0, outgoing, I, and absorbed, I_A, in a cell of length *d.*

The increment *I* photons per unit and time are absorbed in a volume of thickness, x, and area *A*. Each molecule, randomly oriented, has an average cross-section, σ (may be considered the electronic cloud exposed to the photon). The absorption cross-section, *k*, is the product of the probability of absorbing the photon, *p*, and the average cross-section, σ.

Lakowicz, Principles Fluorescence Spectroscopy 3rd Ed., 2006

Spectroscopy



absorption cross-section:

$k = p\,\sigma$; [cm^2]

The incremental intensity absorbed per unit volume element $A\,\Delta x$:

$$\Delta I\,\Delta x \quad = -knI$$

n is the number of molecules and I is the incident light intensity

In the integrated form, where $I = I_0$ at x = 0, then $I/I_0 = \exp(-knd)$

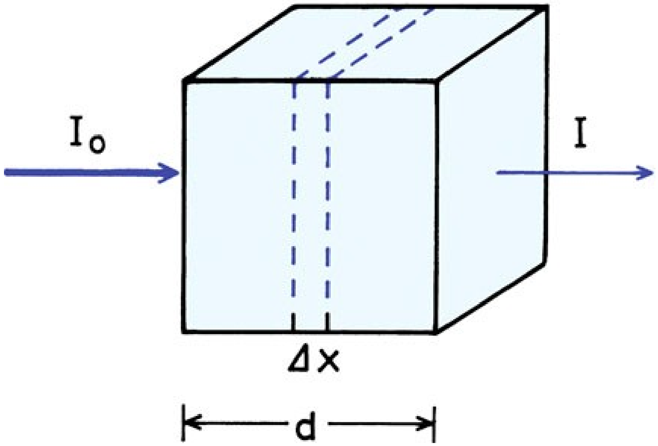
$$\ln (I_0/I) = knd;\ \text{or}\ \log (I_0/I) = \varepsilon cd = \text{OD}$$

c is the concentration, [M]; ε is the extinction coefficient, [M^{-1} cm^{-1}];

and OD is the optical density.

Lakowicz, Principles Fluorescence
Spectroscopy 3rd Ed., 2006

Spectroscopy



absorption cross-section:

$$k = p\,\sigma;\ [\mathrm{cm}^2]$$

One can then see the relationship between absorption cross-section and extinction coefficient:

$$k = 2.303\,(\varepsilon\, c / n)$$

Because $n = N\,c / 10^3$ (where N is Avogadro's number and n is the number of molecules in 1 mL), it turns out that $k = 3.82\ \ 10^{-21}\,\varepsilon$ (where the cross-section is in cm^2 per molecule)

Lakowicz, Principles Fluorescence
Spectroscopy 3rd Ed., 2006

Spectroscopy

Consider what this means: the extinction coefficients of anthracence at 253 and 375 nm are 160,000 and 6,300 M^{-1} cm^{-1}, respectively.

160,000 M^{-1} cm^{-1} x 3.82 x 10^{-21} gives an absorption cross-section of 6.1 x 10^{-16} cm^2 or 6.1 $Å^2$. Similarly, 6,300 M^{-1} cm^{-1} gives an absorption cross-section of 0.24 $Å^2$.

If we assume that anthracene has a molecular cross-section of ~ 12 $Å^2$, then at 253 nm it absorbs ~ 50% of the photons and at 375 nm it absorbs about 2% of the photons. But remember, the orientation is random in a solution. What does this do to the estimate?

Lakowicz, Principles Fluorescence Spectroscopy 3rd Ed., 2006

Spectroscopy

Significance of deviations from the Beer-Lambert Law:

If nothing happens in a solution as a function of concentration of the component solutes, that is there are no concentration-dependent interactions, then the OD should increase linearly with increases in concentration of the components (this requires that all solute components have proportional increases in concentration or that different components have equivalent extinction coefficients.

Deviations mean interactions. The change in absorbance versus concentration will follow the thermodynamics of the system, reflecting cooperativity, positive or negative, or lack thereof.

Spectroscopy

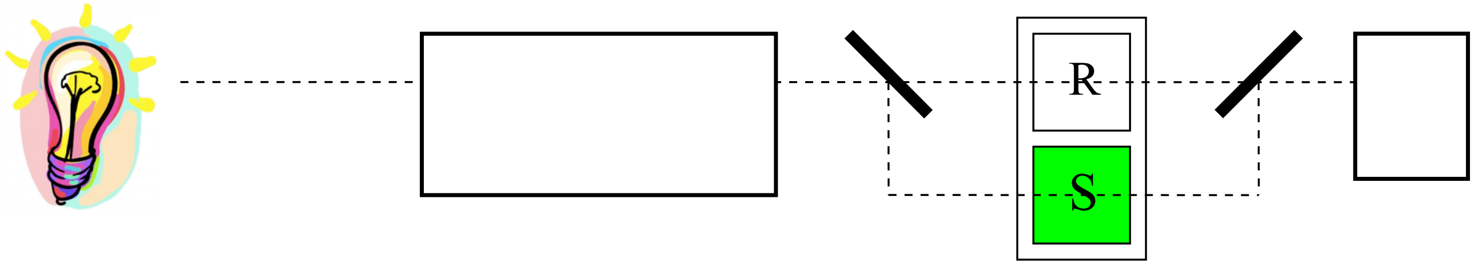
Absorption

Reported by:

1) percent transmission ($\%T_\lambda = 100 \times I_\lambda / I_{0,\lambda}$)

2) absorbance ($A_\lambda = \log \{I_{0,\lambda}/I_\lambda\} = \varepsilon_\lambda \, c \, l$)

Measured by: single or double beam spectrophotometer



light source monochromator cuvette holder detector