UV/Visible spectroscopy

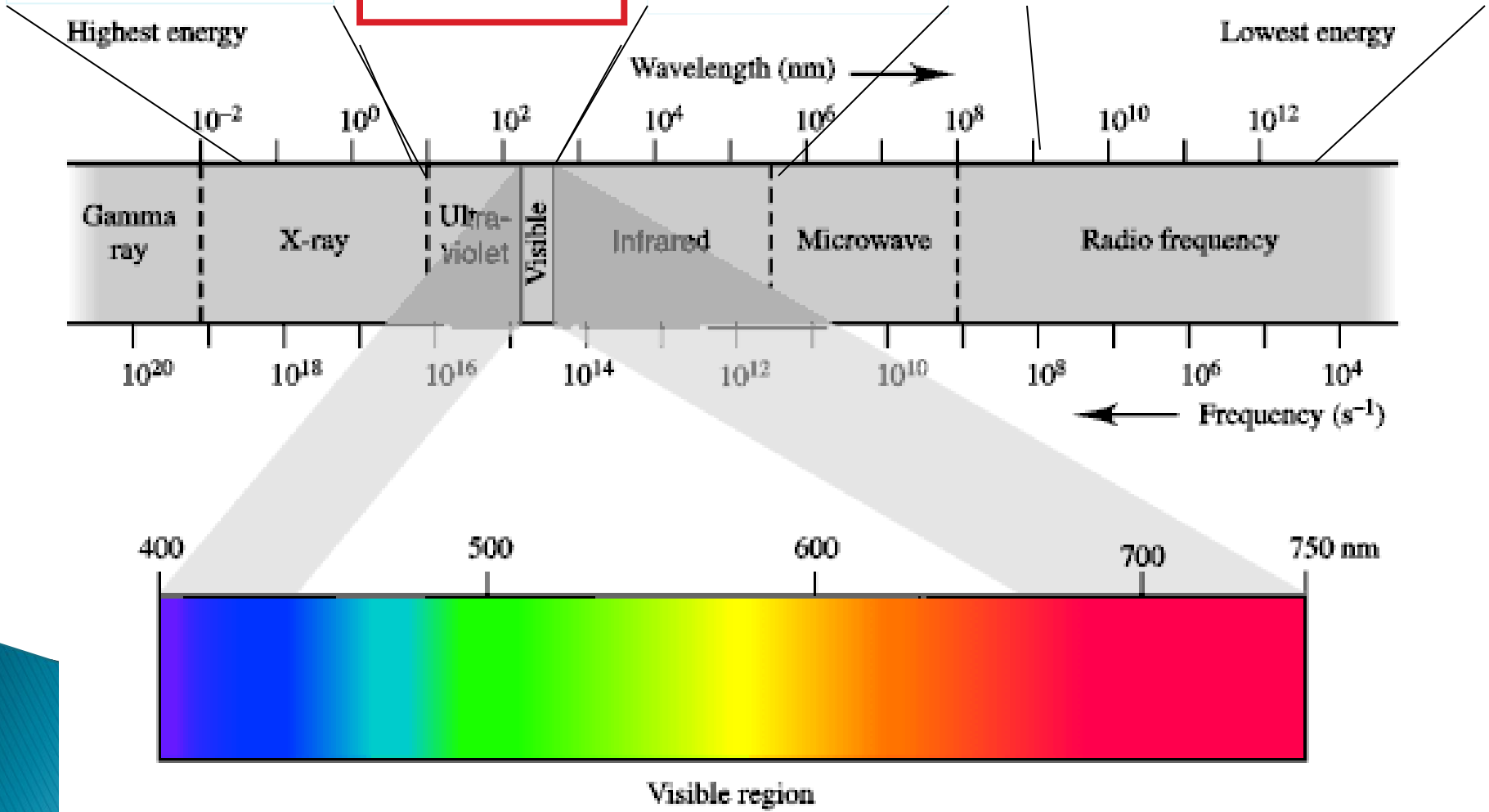
Electronic Excitation by UV/Vis Spectroscopy :

X-ray: core electron excitation

UV/vis: electronic excitation

IR: molecular vibrations

Radio waves: Nuclear spin states (in a magnetic field)



- Many molecules have chromophores that absorb UV
- Involves electronic transitions
- Useful because timescale is so fast, and sensitivity high.

e.g: wavelength 300 nm $\approx 10^{15}$ s^{-1} frequency

Time for absorption $\approx 10^{-15}$ s time scale

- Kinetics, esp. in biochemistry, enzymology

Rate constant determination

- Plot of Absorbance Vs Time

- Absorbance of light at a given wavelength is the sum of the absorbance of the different complex ions in solution. It can be seen that

A_0–initial absorbance (t=0),
A –final absorbance, at infinite time.
c –concentration at time t
c_0 –initial concentration

$$\frac{c}{c_0} = \frac{A - A_\infty}{A_0 - A_\infty}$$

- A plot of ln [A – A / A_0 – A] vs. time will give a straight line with slope –k, for a first order or pseudo first order reaction.

A common use: Enzyme kinetics

E.g: Effect of enzyme concentration on rate of reaction

- Enzyme assay of varying enzyme concentrations
- Absorbance measured at wavelength of maximum absorbance
- Plot of Absorbance Vs Concentration to compute ϵ. ($A = \epsilon C l$)
- Calculate and plot the reaction rate as a function of enzyme concentration.