Lecture 1
Spectroscopy

Introduction

- One of the earliest experimental measurements on biomolecules involved studies of their interactions with electromagnetic radiation of all wavelengths, including X-ray, ultraviolet–visible, and infrared.

- It was experimentally observed that when light impinges on solutions or crystals of molecules, at least two distinct processes occur:
  - ✓ light scattering and
  - ✓ light absorption

- Both processes have led to the development of fundamental techniques for characterizing and analyzing biomolecules.

- Absorption of ultraviolet–visible light by molecules is an especially valuable process for measuring concentration and for molecular structure elucidation.

- The absorption process is dependent upon two factors:
  - ✓ the properties of the radiation (wavelength, energy, etc.), and
  - ✓ the structural characteristics of the absorbing molecules (atoms, functional groups, etc.)

- The interaction of electromagnetic radiation with molecules is a quantum process and described mathematically by quantum mechanics; that is, the radiation is subdivided into discrete energy packets called photons. In addition, molecules have quantized excitation levels and can accept packets of only certain quantities of energy, thus allowing only certain electronic transitions.

- With some molecules, the process of absorption is followed by emission of light of a longer wavelength. This process, called fluorescence, depends on molecular structure and environmental factors and assists in the characterization and analysis of biologically significant molecules and dynamic processes occurring between molecules.

- Radiations are composed of two components i.e.,
  - ✓ electric and
  - ✓ magnetic vector.
    - The two components are perpendicular to each other and to the direction of propagation.
    - The wave nature of these radiations is generally specified by three physical properties including frequency (f), wavelength (λ), or photon energy (E).
- Wavelength is the distance between two consecutive peaks measured in nanometers (nm).
- Maximum length of the vector is called the amplitude.

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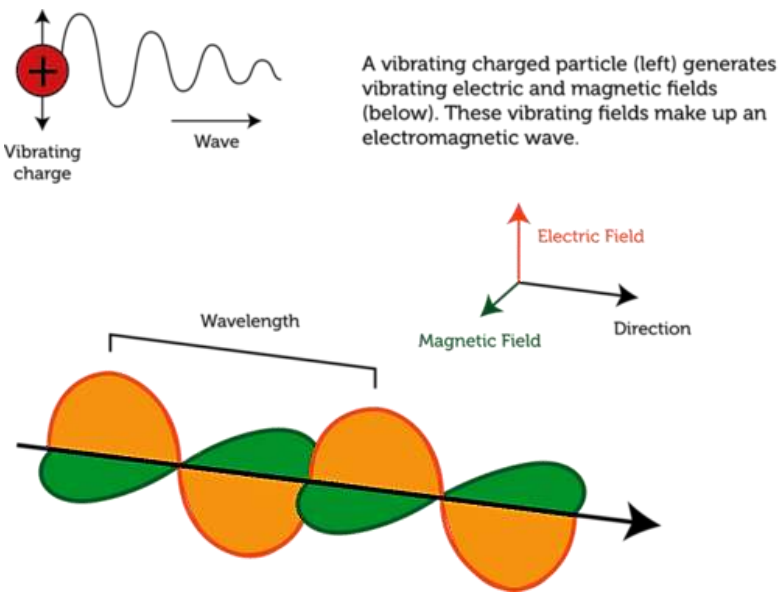
- Frequency of the electromagnetic radiation is the number of oscillations made by the wave within the time frame of 1 second (units of 1/s).

- Wavenumber describes the number of completed wave cycles per distance and is typically measured in 1/cm.



Interaction of Electromagnetic Radiation with Matter

- In biochemistry, the "matter" refers to the biologically important molecules or biomolecules.

- In this chapter interaction of electromagnetic radiation with matter refers to
  - ✓ amino acids
  - ✓ peptides
  - ✓ proteins,
  - ✓ carbohydrates
  - ✓ nucleic acids (DNA, RNA)
  - ✓ lipids
  - ✓ vitamins
  - ✓ hormones etc.

- The interaction of electromagnetic radiation with matter is a quantum phenomenon and dependent upon both the properties of the radiation and the appropriate structural parts of the samples involved.

- This is not surprising, since the origin of electromagnetic radiation is due to energy changes within matter itself.

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- The transitions which occur within matter are quantum phenomena and the spectra which arise from such transitions are principally predictable.

- The photon is the elementary particle responsible for electromagnetic phenomena. It carries the electromagnetic radiation and has properties of a wave, as well as of a particle, albeit having a mass of zero. As a particle, it interacts with matter by transferring its energy E.

E	=	hc / λ
	=	hν
h	=	Planck constant ($h = 6.63\times10^{-34}$ Js)
ν	=	Frequency of the radiation.

- When a photon of electromagnetic radiation interacts with a molecule, there are two possibilities, the first one is the scattering of light also known as Rayleigh scattering that occurs when the photon interacts with the molecules and is then diffracted or scattered with unchanged frequency.

- This phenomenon is the basis for various techniques like
  - X-ray crystallography,
  - electron microscopy,
  - laser light scattering and
  - neutron scattering.

  In biochemistry these techniques have been by exploited by various researchers for identification and characterization of numerous biomolecules.

- The second phenomenon is the absorption of the light that occurs when the photon of light transfer its energy to the molecule, inducing an excited state of the molecules.

- This process is known as absorption spectrum and is the main mechanism behind the UV-Visible spectroscopy.

- When the light of certain energy is incident of the solution with some molecules, its electrons are excited from the ground (G) state to the excited sate (S1). However for the photon to be absorbed, its energy must match the energy difference between two energy levels of the molecule. When electrons are shifted from the ground state to excited sate an energy transition is said to occur. The energy associated with the ultraviolet and visible light is sufficient to promote molecules from one electronic state to another, that is, to move electrons from one orbital to another.

- Within each electronic energy level is a set of vibrational energy levels. These represent changes in the stretching and bending of covalent bonds. The transition between these energy levels is the basis of the infrared spectroscopy.

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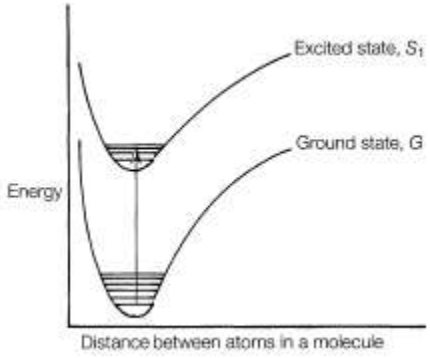
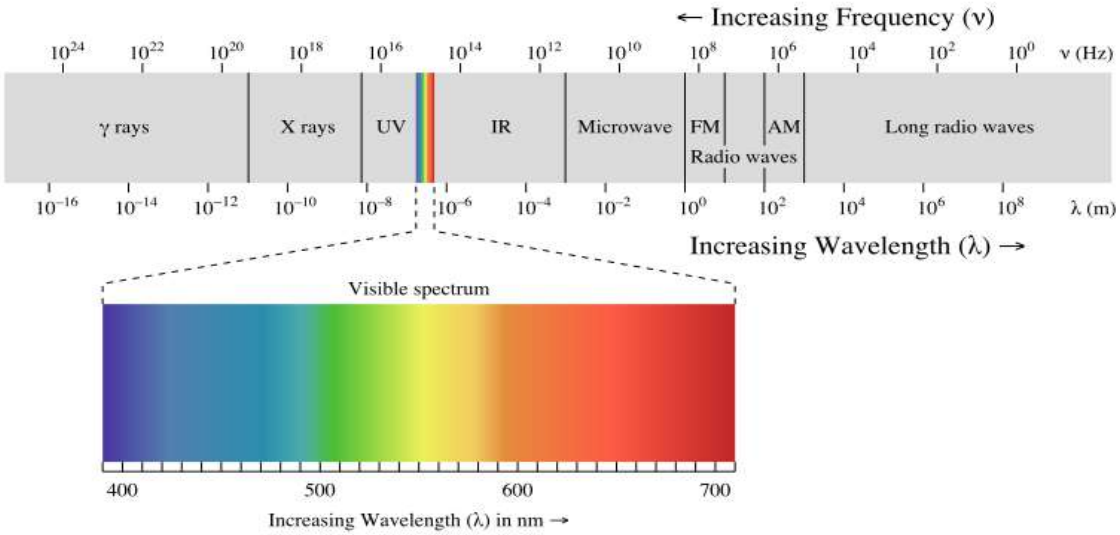


Types of Electromagnetic Spectrum

- As a wave's wavelength increases, the frequency decreases, and vice versa.
- When electromagnetic energy is released as the energy level increases, the wavelength decreases and frequency decreases.
- Thus, electromagnetic radiation is then grouped into categories based on its wavelength or frequency into the electromagnetic spectrum.
- The different types of electromagnetic radiation shown in the electromagnetic spectrum consists of radio waves, microwaves, infrared waves, visible light, ultraviolet radiation, X-rays, and gamma rays.
- The part of the electromagnetic spectrum that we are able to see is the visible light spectrum.



Name of spectroscopy	Radiation	Wave Length	Energy	Effect on electrons or atom	Information regarding atom/molecule
Gamma rays spectroscopy (Positron Emission spectroscopy)	Gamma -rays	0.0001-0.01nm	Very high	Removes core electrons	Imaging of the body inside treat of malignant tumors by radiotherapy

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Photoelectron spectroscopy (PES)	X-rays	0.01 to 10 nm	Very high	Removes core electrons	Atomic structure How tightly the electrons are held by the nucleus Identity of an element
UV-spectroscopy	Ultraviolet	50-400 nm	High	Excites valence electrons	Identity of a molecule or element Quantification of biomolecules
Visible spectroscopy	Visible	400-800 nm	Medium	Excites valence electrons	Quantification of biomolecules
Infrared (IR) spectroscopy	infrared	2.5-50 nm	Low	Changes the vibrations in covalent bonds	Types of bonds or atoms of functional groups within a molecule
Microwave (rotational) spectroscopy	Microwave	0.3nm-0.5nm	Very low	Changes the rotations of the atoms in covalent bonds	Location of hydrogen atoms within a molecule