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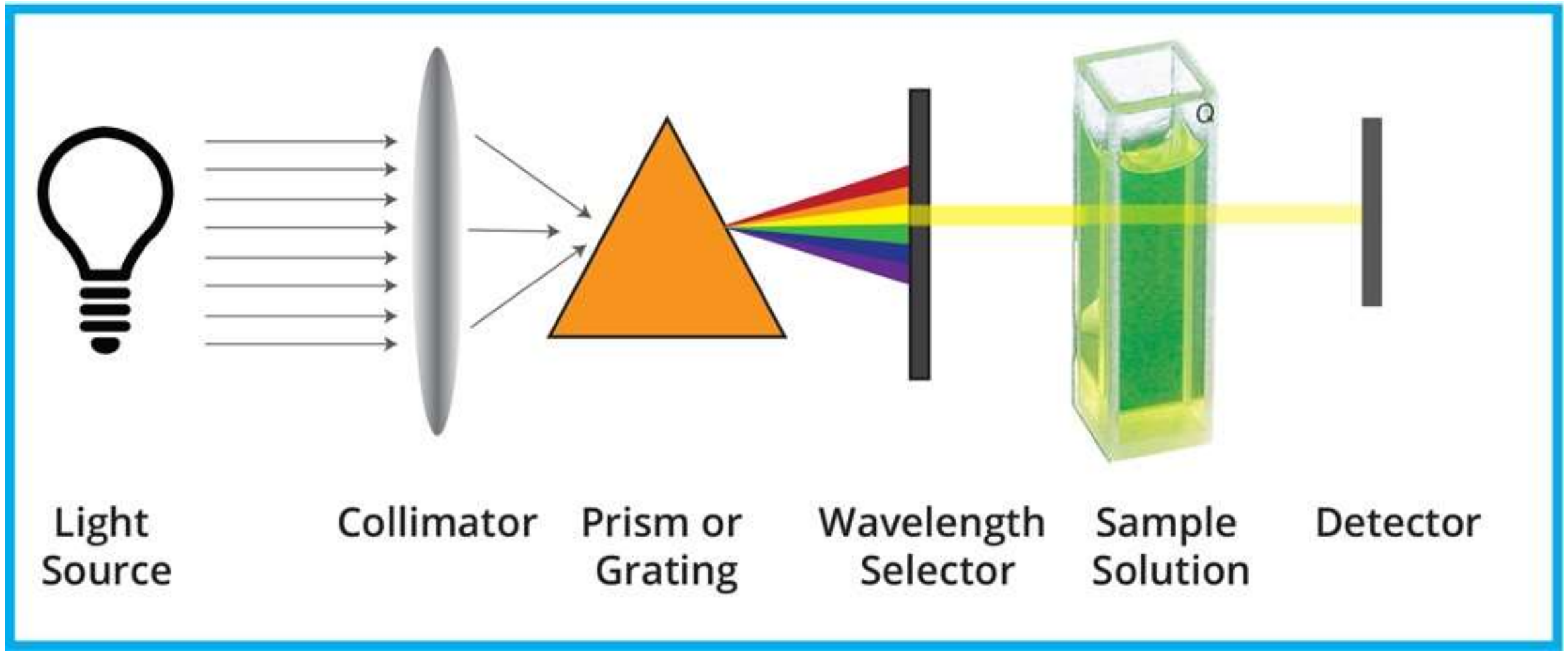
# UV/Visible Light Spectroscopy



# Introduction

- The electronic transitions in molecules can be classified according to the participating molecular orbitals.
- From the four possible transitions (n → $\pi^*$, $\pi$ → $\pi^*$, n → $\sigma^*$, $\sigma$ → $\sigma^*$), only two can be elicited with light from the UV/Vis spectrum for some biological molecules: n → $\pi^*$ and $\pi$ → $\pi^*$.

# Chromophores

- Molecular (sub-)structures responsible for interaction with electromagnetic radiation are called chromophores.
- In proteins, there are three types of chromophores relevant for UV/Vis spectroscopy:
- Peptide bonds (amide bond);
- Certain amino acid side chains (mainly tryptophan and tyrosine); and
- Certain prosthetic groups and coenzymes (e.g. porphyrin groups such as in heme).

# Peptide Bond as Chromophore

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- The electronic transitions of the peptide bond occur in the far UV.
- The intense peak at 190 nm, and the weaker one at 210–220 nm is due to the n $\rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions.
- A number of amino acids (Asp, Glu, Asn, Gln, Arg and His) have weak electronic transitions at around 210 nm.
- Usually, these cannot be observed in proteins because they are masked by the more intense peptide bond absorption.

# Side Chain Group as Chromophore

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- The most useful range for proteins is above 230 nm, where there are absorptions from aromatic side chains.
- While a very weak absorption maximum of phenylalanine occurs at 257 nm, tyrosine and tryptophan dominate the typical protein spectrum with their absorption maxima at 274nm and 280 nm, respectively.
- Cystine ($Cys_2$) possesses a weak absorption maximum of similar strength as phenylalanine at 250 nm.
- This band can play a role in rare cases in protein optical activity or protein fluorescence.

# Prosthetic Groups as Chromophore

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- Proteins that contain prosthetic groups (e.g. heme, flavin, carotenoid) and some metal–protein complexes, may have strong absorption bands in the UV/Vis range.
- Carotenoids, for instance, are a large class of red, yellow and orange plant pigments.
- They contain three maxima in the visible region of the electromagnetic spectrum (420 nm, 450 nm, 480 nm).
- Porphyrins are the prosthetic groups of hemoglobin, myoglobin, catalase and cytochromes.

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- Electron delocalization extends throughout the cyclic tetrapyrrole ring of porphyrins and gives rise to an intense transition at 400nm called the Soret band.
- Molecules such as FAD (flavin adenine dinucleotide), NADH and $NAD^+$ are important coenzymes of proteins involved in electron transfer reactions (Redox reactions).
- They can be conveniently assayed by using their UV/Vis absorption: 438nm (FAD), 340nm (NADH) and 260nm ($NAD^+$).

# Chromophores in Genetic Material

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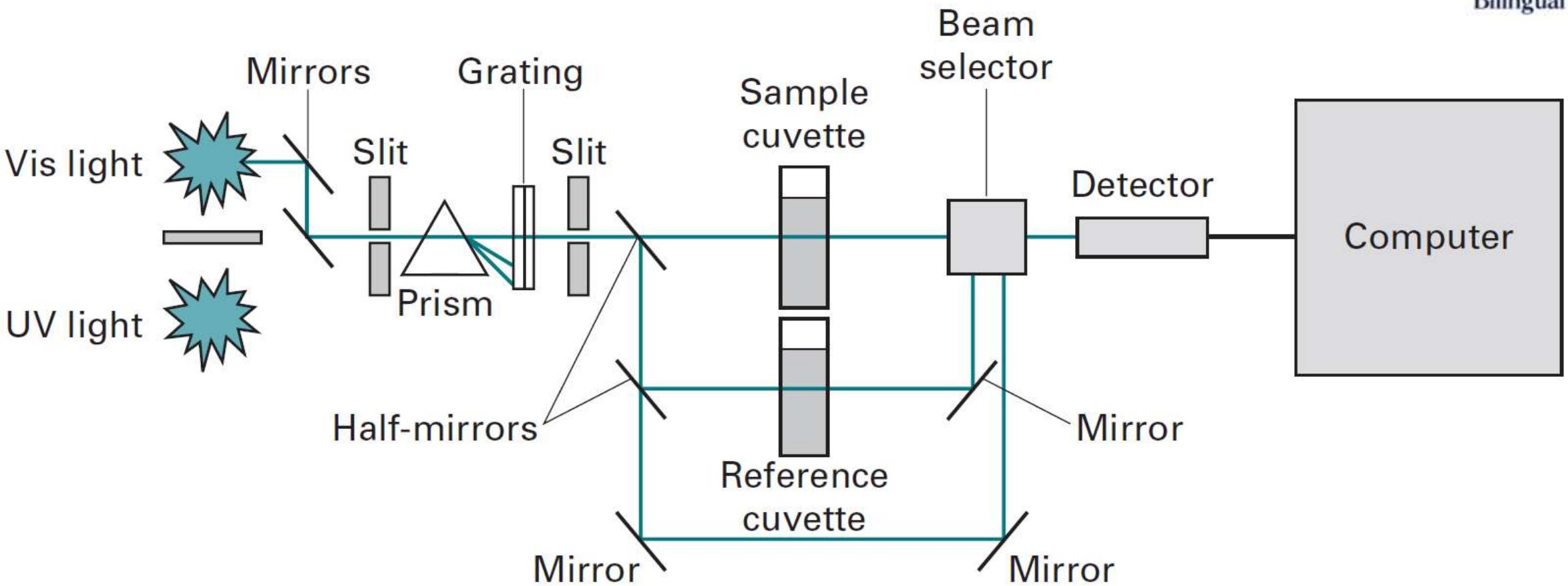
- The absorption of UV light by nucleic acids arises from n → $\pi^*$ and $\pi$ → $\pi^*$ transitions of the purine (adenine, guanine) and pyrimidine (cytosine, thymine, uracil) bases that occur between 260nm and 275 nm.
- The absorption spectra of the bases in polymers are sensitive to pH and greatly influenced by electronic interactions between bases.



# Beer's Law

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- When monochromatic light passes through a solution there is usually a quantitative relationship between the solute concentration and the intensity of the transmitted light
- The amount of light absorbed by the a medium (solution/ sample) is proportional to the concentration of the absorbing material or solute present.
- Thus the concentration of a solute in a solution may be determined in the lab by measuring the **absorbance of light at a given wavelength**

# Lambert's Law

- Lambert described how intensity changes with distance in an absorbing medium.
- The amount of light absorbed by the medium ( solution/sample) at a given wavelength is proportional to the thickness of the absorbing layer: path length of the light

# Beer Lambert Law

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- The chance for a photon to be absorbed by matter is given by an extinction coefficient which itself is dependent on the wavelength λ of the photon.
- If light with the intensity $I_0$ passes through a sample with appropriate transparency and the path length (thickness) *d*, the intensity *I* drops along the pathway in an exponential manner.
- The characteristic absorption parameter for the sample is the extinction coefficient α, yielding the correlation $I = I_0 e^{-\alpha d}$.
- The ratio $T = I/I_0$ is called transmission.

- Biochemical samples usually comprise aqueous solutions, where the substance of interest is present at a molar concentration ***c***.
- Algebraic transformation of the exponential correlation into an expression based on the decadic logarithm yields the law of Beer–Lambert.
- $\log I_0/I = \log 1/T = \varepsilon \times c \times d = A$
- where $[d] = 1\text{cm}$, $[c] = 1\text{mol dm}^{-3}$, and $[\varepsilon] = 1\text{dm}^3\text{mol}^{-1}\text{ cm}^{-1}$.
- $\varepsilon$ is the molar absorption coefficient (also molar extinction coefficient).
- *A* is the absorbance of the sample, which is displayed on the spectrophotometer.
- The Beer–Lambert law is valid for low concentrations only.
- Higher concentrations might lead to association of molecules and therefore cause deviations from the ideal behaviour.

# Auxochromes

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- Auxochromes is any moiety which does not show any colour but when attached to some chromophore it enhances the colour production by forming a new chromophore.
- Auxochromes are functional groups attached to chromophore that alter the shade of colour it emits.
- Example: -OH and —$NH_2$ on benzene chromophore.

- Four effects, two each for wavelength and absorption changes, have to be considered:
  - a wavelength shift to higher values is called red shift or bathochromic effect;
  - similarly, a shift to lower wavelengths is called blue shift or hypsochromic effect;
  - an increase in absorption is called hyperchromicity ('more colour'),
  - while a decrease in absorption is called hypochromicity ('less colour').

# Applications

- Concentration analysis.
- Equilibrium constant of reactions involving ions.
- Absorption, Transmission, etc.
- Molar extinction coefficient of amino acid chain.
- Qualitative and Quantitative analysis.

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