BIOENGINEERING LABORATORY I

Experiment 7

Molecular Fluorescence Spectroscopy and Quantitation of BSA

Dec 2020, Istanbul

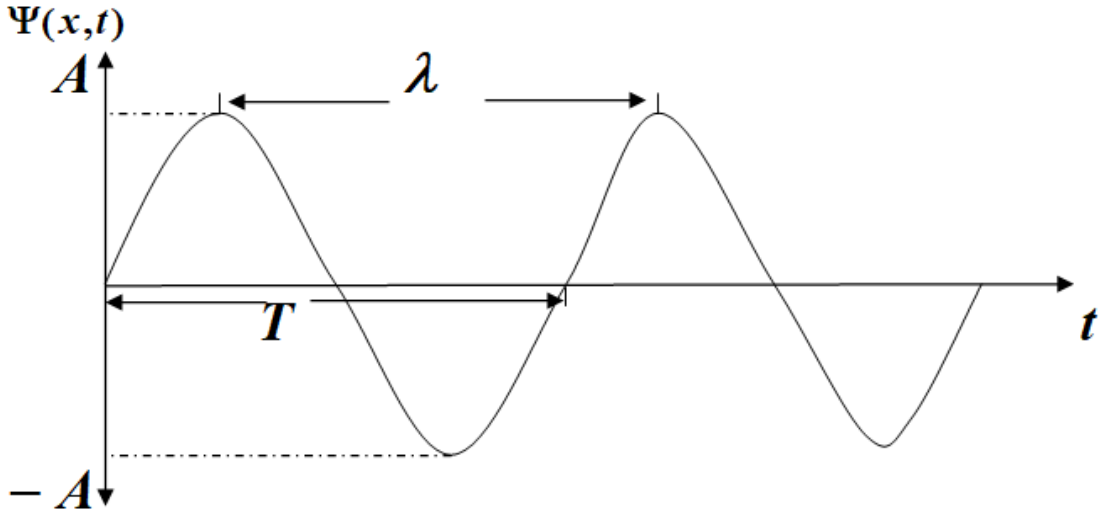
Spectroscopy

- experimental technique that studies the interactions between the electromagnetic wave and the molecules.
- experimental techniques used to determine the electronic structures of atoms.

Spectroscopy Methods

- UV-Visible Spectroscopy
- Atomic Absorbance Spectroscopy
- IR (infrared) spectroscopy
- Mass Spectroscopy
- NMR (Nuclear Magnetic Resonance) Spectroscopy
- **Fluorescence and Phosphorescence Spectroscopy**

ELECTROMAGNETIC WAVE

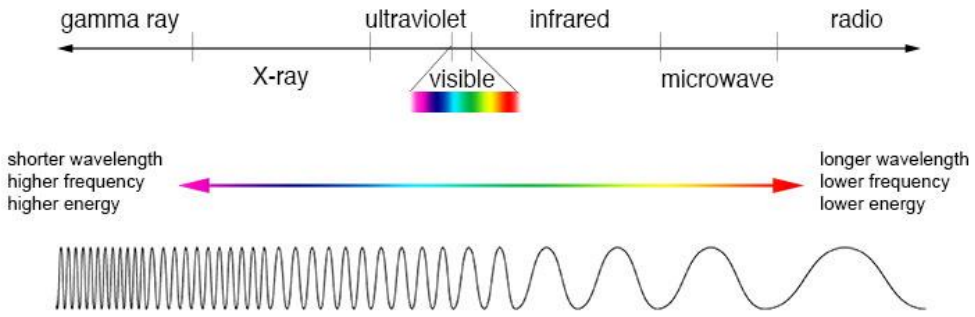


$$f = \frac{v}{\lambda}.$$

- WAVE LENGTH :(λ) The distance between two consecutive maxima (or two minima) in the wave motion of the ray.
- PERIOD: (***T***) It is the time required to repeat the wave motion of the ray.
- WIDTH: (***A***) It is the distance from the maximum point of the wave motion of ray to the horizontal axis.
- FREQUENCY ($\boldsymbol{\nu}$) It is the number of waves passing from a point in a unit time.

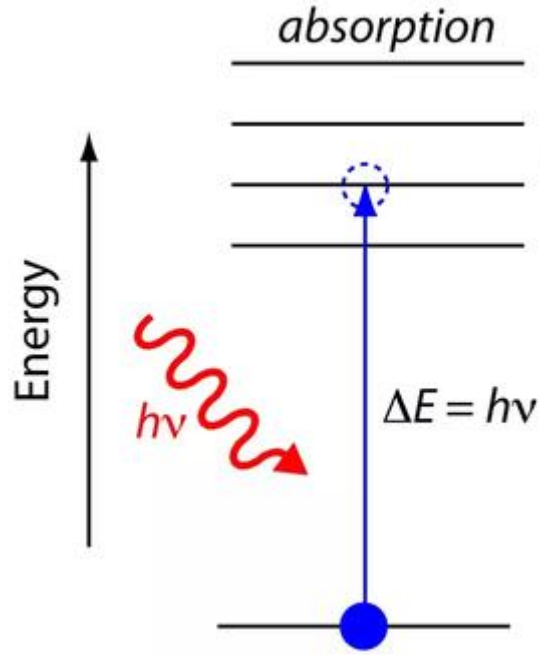
Ray - Matter Interaction

- The ray entering between the surfaces of matter interacts with the atoms and molecules, can be **passed, held or scattered** according to the properties of the substance.
- Electromagnetic radiation is a type of energy that passes through the space at very high speeds, it covers a wide range of wavelengths (energy). **Their interactions with matter are different depending on their frequency.**



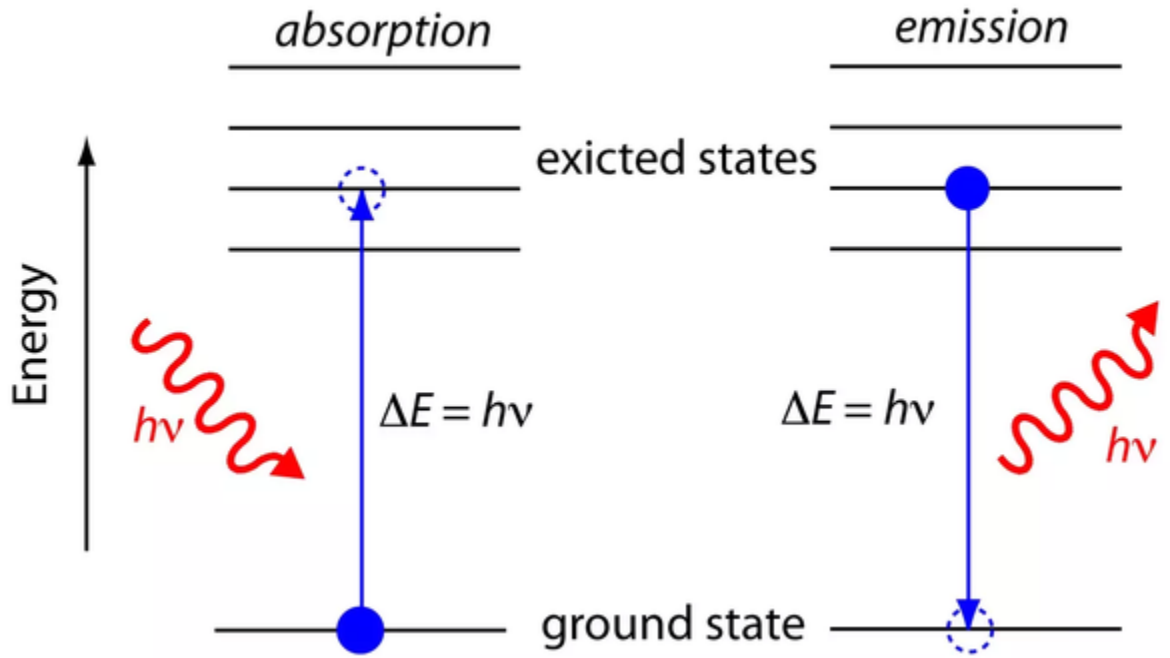
Ray - Matter Interaction: Absorption

- If an electromagnetic wave containing rays of various wavelengths is passed through a transparent medium, the loss of some wavelengths 9through it is defined as **absorption** .
- By absorption, ray energy is transferred to ions, atoms or molecules of the substance.
- Thus, **ions, atoms or molecules that have** absorbed **the** ray energy become excited.

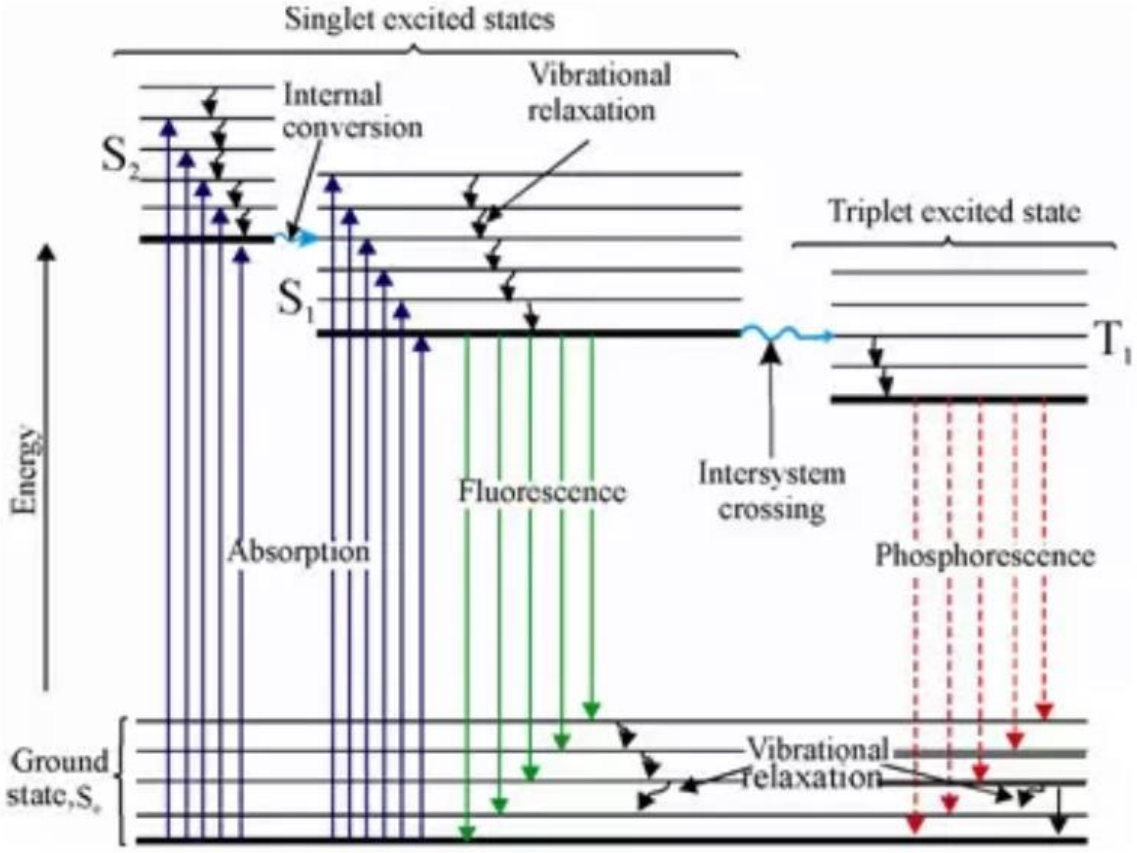


Ray - Matter Interaction: Emission

- **Emission** is the **emission of** a molecule's excess energy as photon while transitioning from a high energy level to a lower energy level .



Excited → Ground State transitions

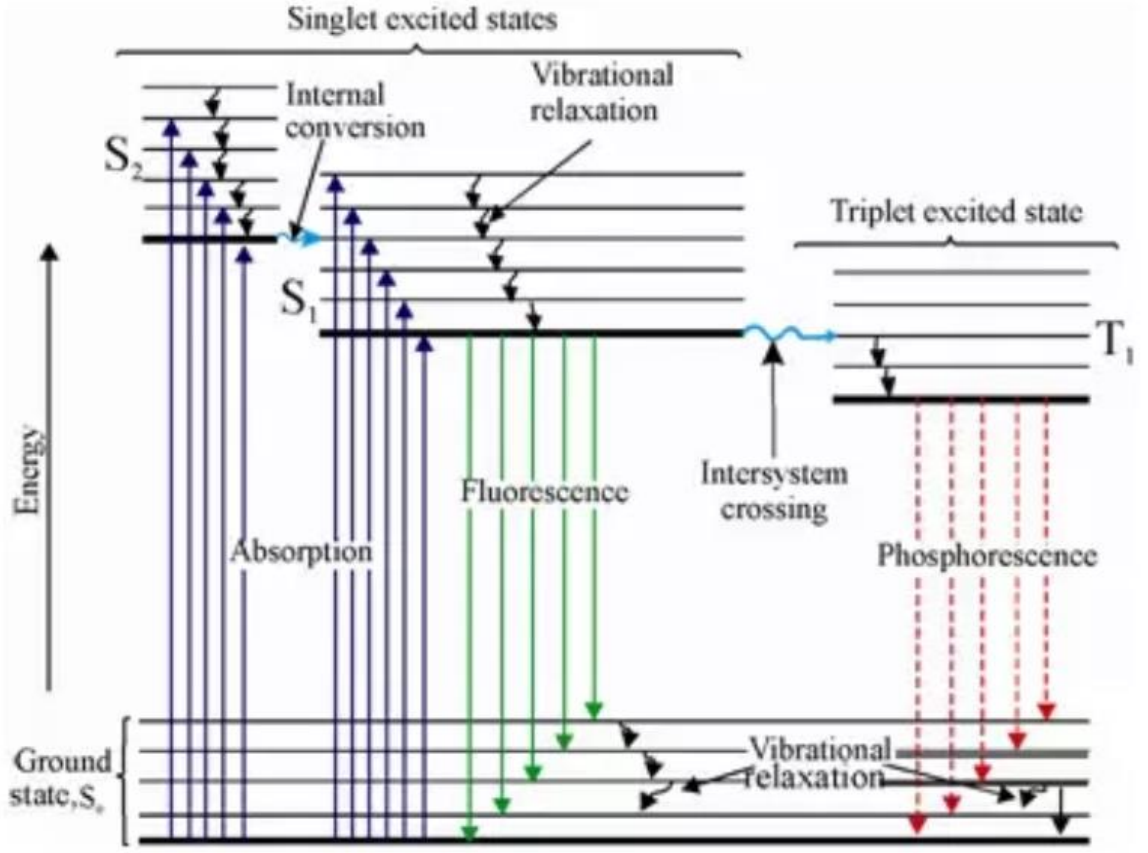


jablonski diagram

Excited → Ground State transitions

Ground State Transition without radiation:

- *Vibrational Relaxation:* It is the transfer of excessive energy of vibrationally excited molecules to solvent molecules.
- *Internal conversion:* It is the transition of an excited molecule from the lowest vibration level of the high electronic level to the upper vibration level of another electronic level of lower energy.

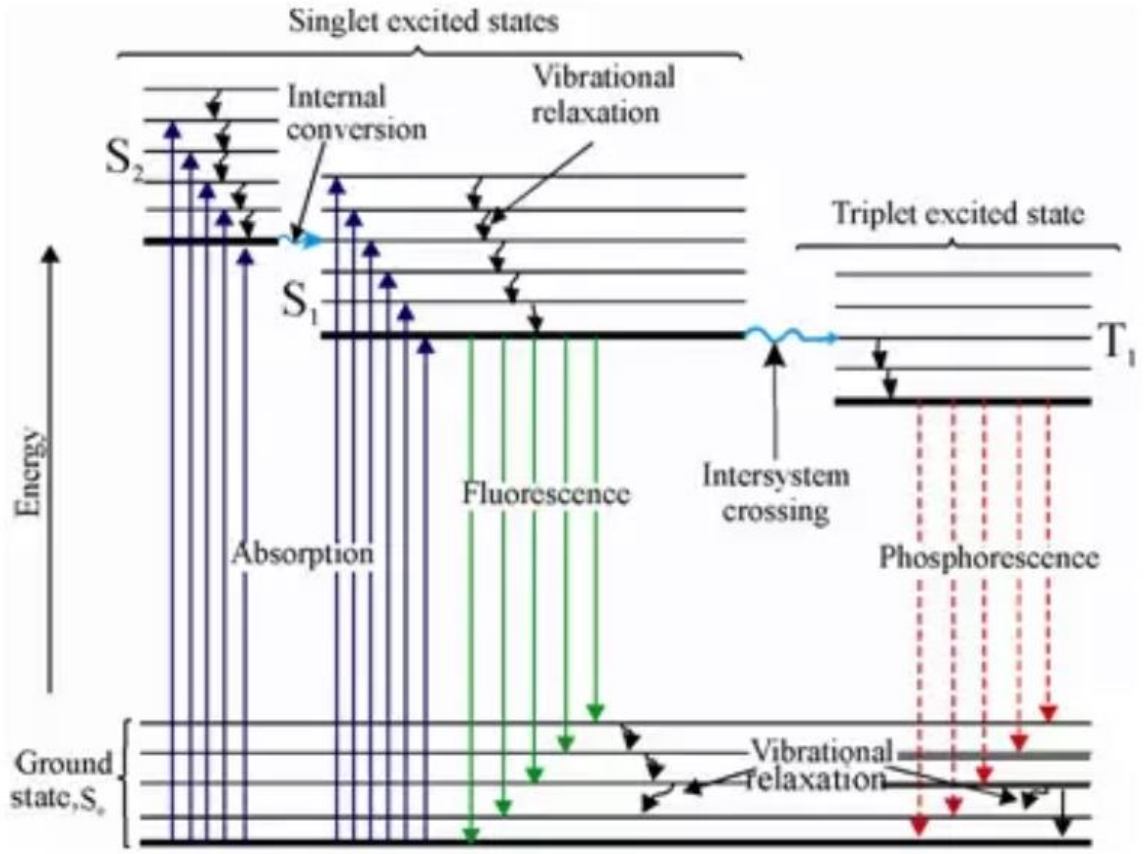


Excited → Ground State transitions

Radiation Ground State Transition (luminescence) **:**

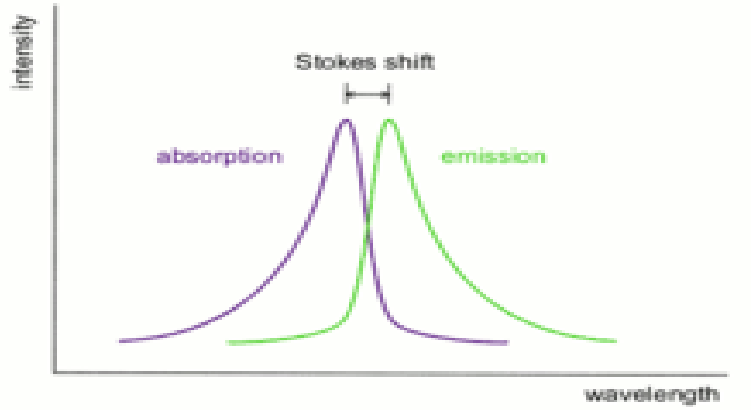
- *Fluorescence*: **It** occurs much faster than phosphorescence and is completed in 10-5 seconds or less after the moment of stimulation
- *Phosphorescence:* It starts in periods greater than 10-5 seconds after the ray ,absorption and may continue for minutes or even hours.
- *Resonance Fluorescence:*

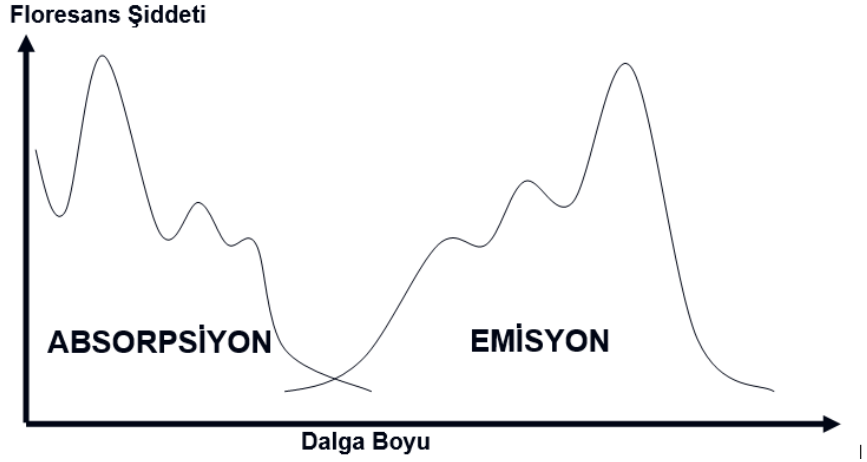
Fluorescence wavelength is the state in which the excitation ray is the same.



Absorption and Emission

- When matter absorbs a photon, it gains energy and enters the excited state. However, in order to become stable, it **gives its energy by** emitting photons, that is **, emitting (radiating, emitting photon). The energy of this emitted photon has less energy than the energy of the absorbed photon. This energy difference is called the Stokes Shift.**



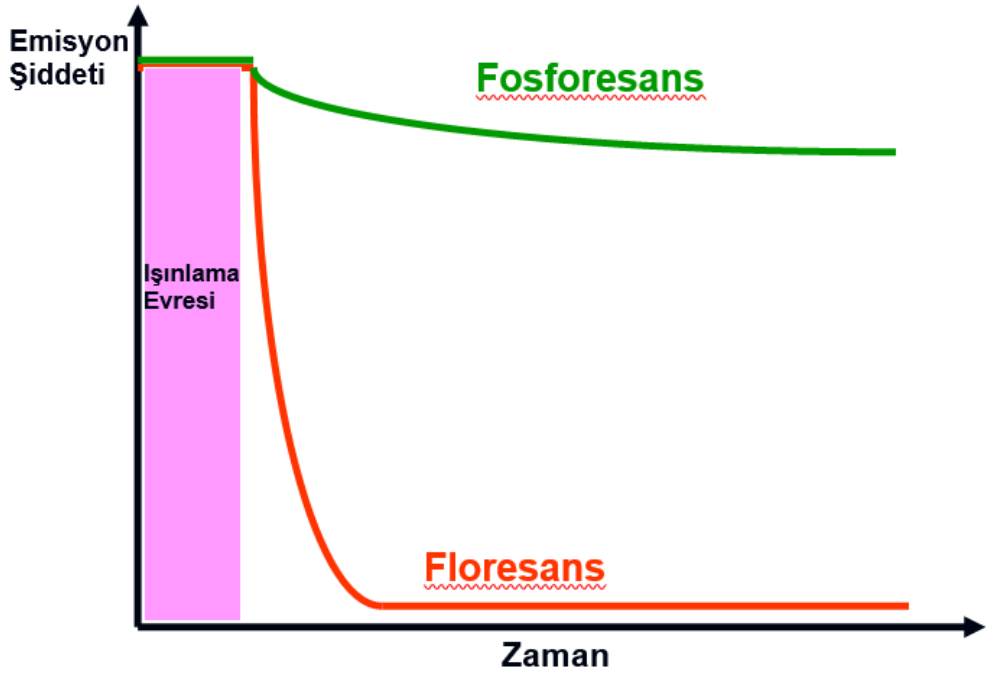


- Absorption and emission spectra are approximately **mirror images** .

Fluorescence and Phosphorescence Spectroscopy

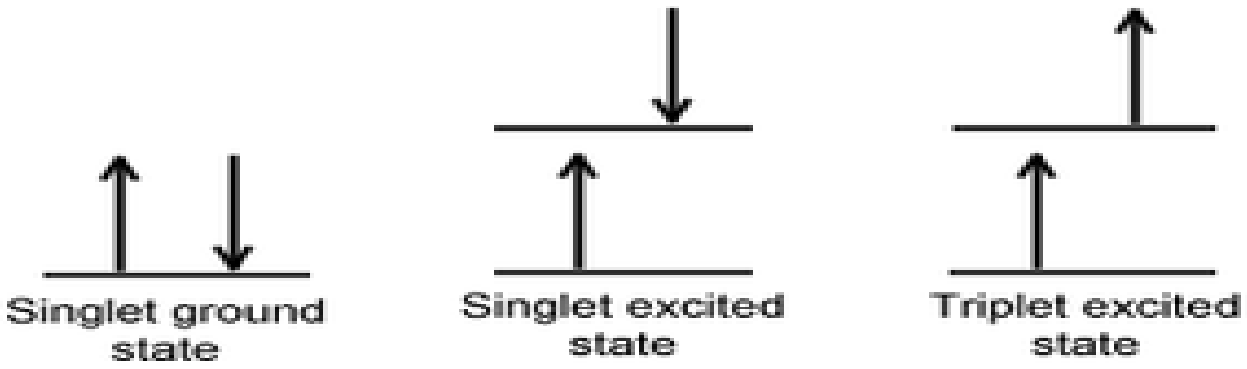
Fluorescence and Phosphorescence

- **LUMINESCENCE:** The material that absorbs the light energy emits longer wavelength rays for a short time.
- **FLUORESCENCE:** It is the situation in which luminescence event occurs in a very short time.
- **PHOSPHORESCENCE :** The situation in which the luminescence event occurs in a longer time.



Fluorescence and Phosphorescence

Fluorescence	Phosphorescence
Spontaneous radiation stops immediately when the exciter ray stops.	Spontaneous radiation may take a long time (minutes).
Absorbed ray is instantly transformed into new another ray	The energy is first stored and slowly emitted from here in the form of rays.
Radiation from the excited singlet to the ground state.	Radiation from excited triplet to ground state



Fluorescence Lifetime and Quantum yield

- The duration of fluorescence material in excited state is defined as the fluorescence **lifetime**.
- **It is the ratio of the number of luminescent molecules to the total number of excited molecules.**
- Under some conditions, the quantum yield for a highly fluorescent molecule such as fluorescein closes to 1.
- Significantly, non-fluorescent chemical species have yields close to 0.

$$Quantum\ Yield = \frac{Number\ of\ emitted\ atom}{Number\ of\ Absorbed\ atom} = \frac{Number\ of\ flourescent\ molecules}{Total\ number\ of\ excited\ molecules}$$

Factors Affecting Fluorescence

- ***Factors related to molecular structure*** **:**
- Molecules that carry aromatic or multiple conjugated double bonds have fluorescence properties.
- The most intense fluorescence are systems with aromatic rings inside.
- Fluorescence is mostly seen in aromatic systems with polycyclic structure.
- If the molecular structure is rigid, its fluorescence increases.

Factors Affecting Fluorescence

Extrinstic Factors:

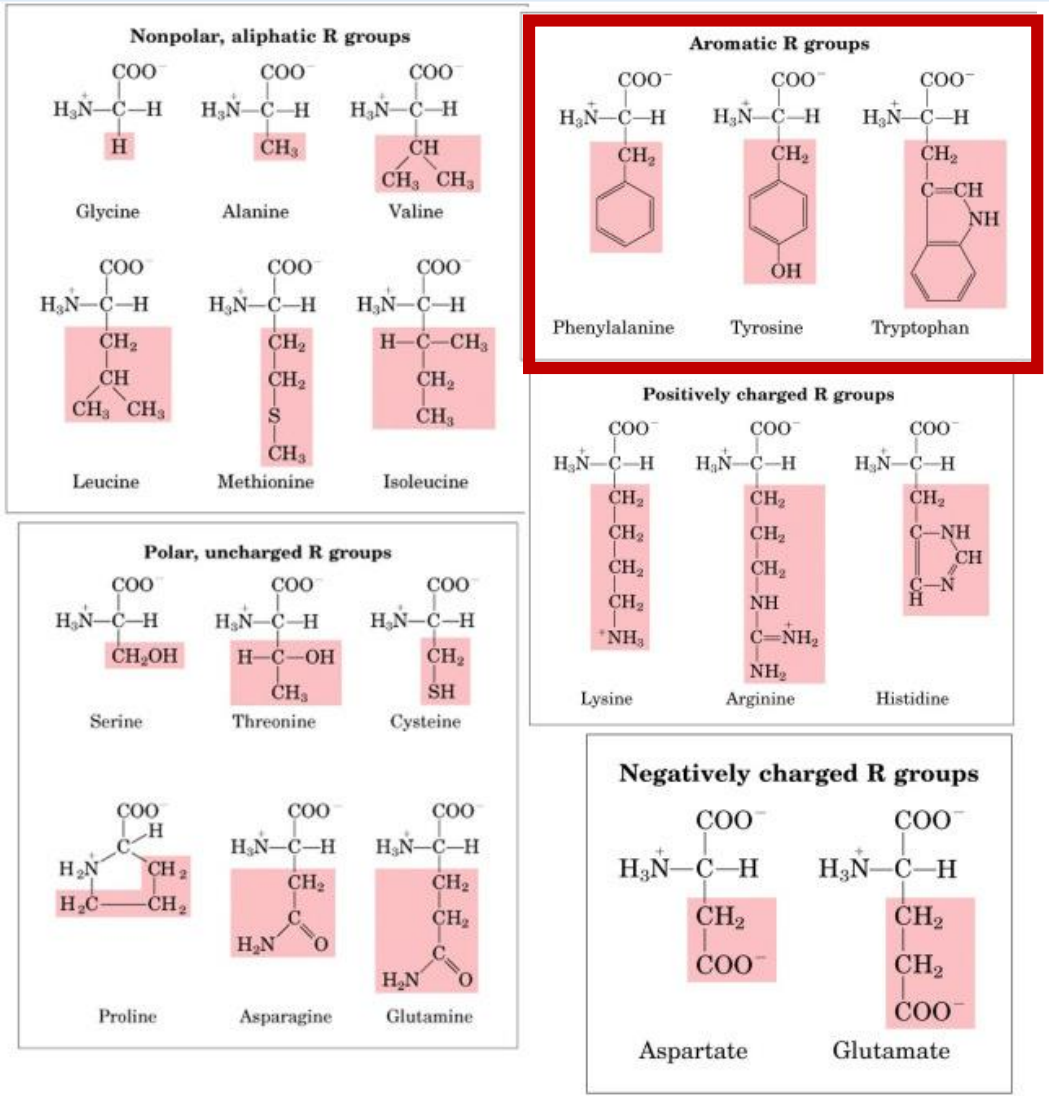
- Fluorescence changes with pH, depending on acid or base group content.
- Increasing temperature decreases fluorescence.
- Increasing the polarity of the solvent also increases the fluorescence.
- Dissolved oxygen generally reduces the fluorescence intensity.
- **The fluorescence intensity is proportional to the concentration of fluorescent agent in solution.**

Usage Areas

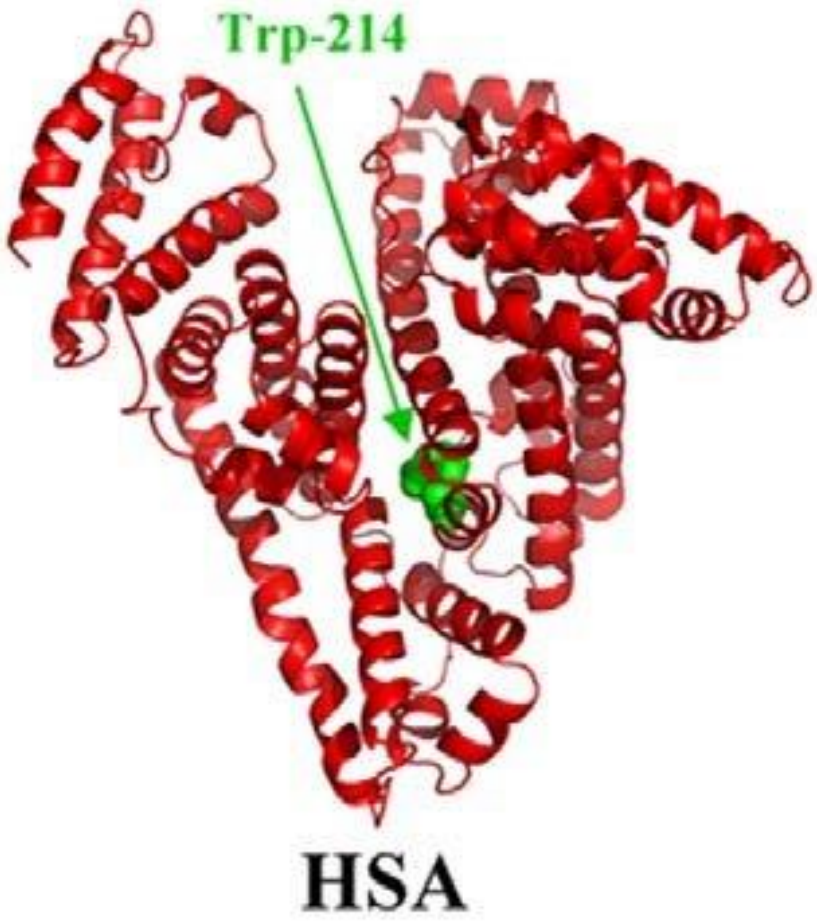
- Intracellular concentration determination,
- Protein quantification,
- Information about its three-dimensional structure (unfolded or folded),
- DNA binding,
- Determination of nutrients, drugs, clinical samples and natural substances.

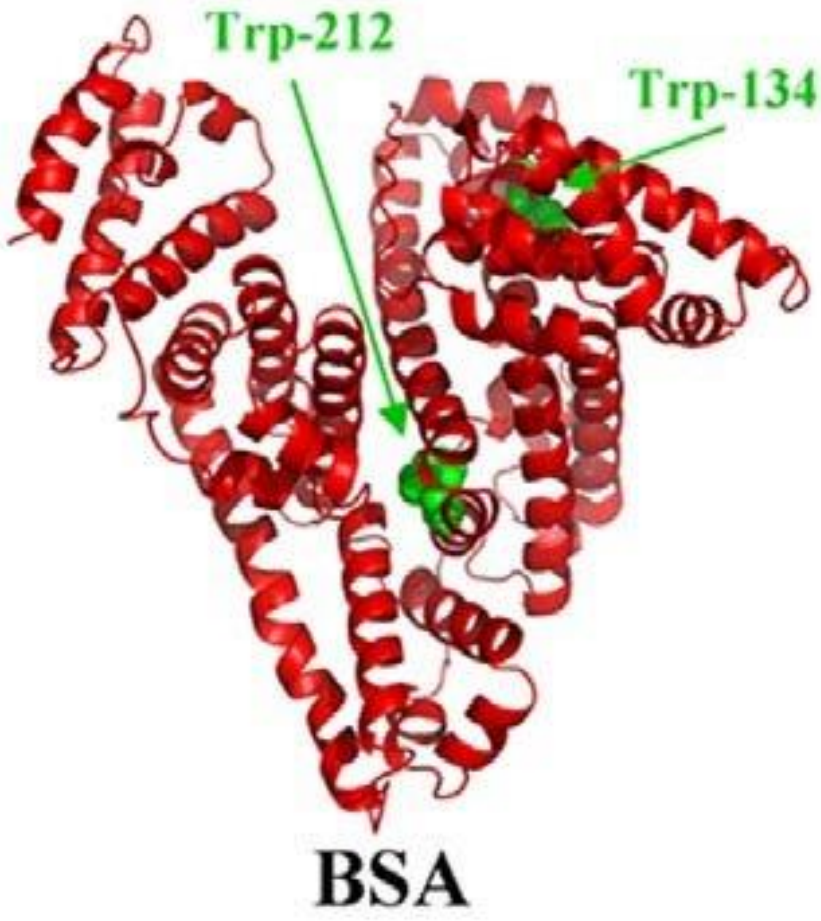
Fluorescent Amino Acids

- Proteins and peptides with aromatic amino acids fluoresce spontaneously when excited by UV light.
- These amino acids have different absorption and emission wavelengths and their quantum efficiencies also differ.



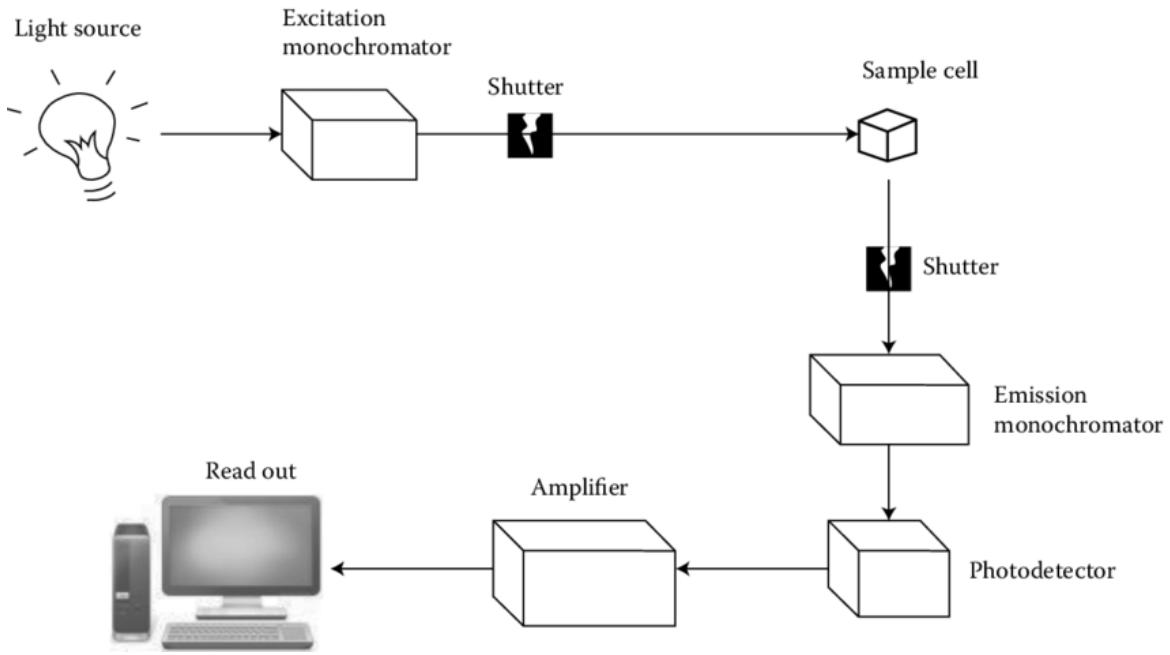
BSA: Bovine Serum Albumin

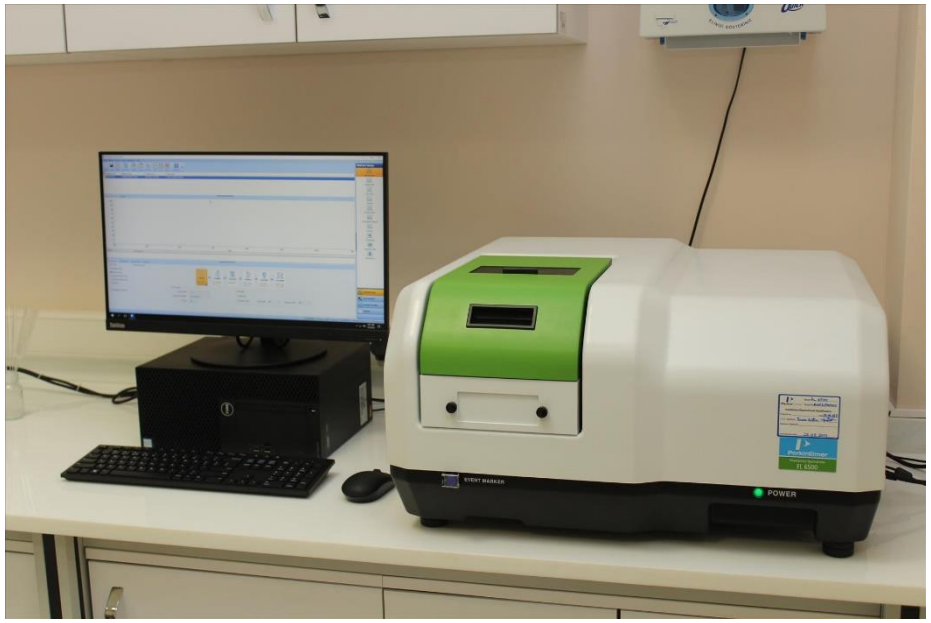




Experimental Method

Schematic Representation of Experimental Setup and Device





Method

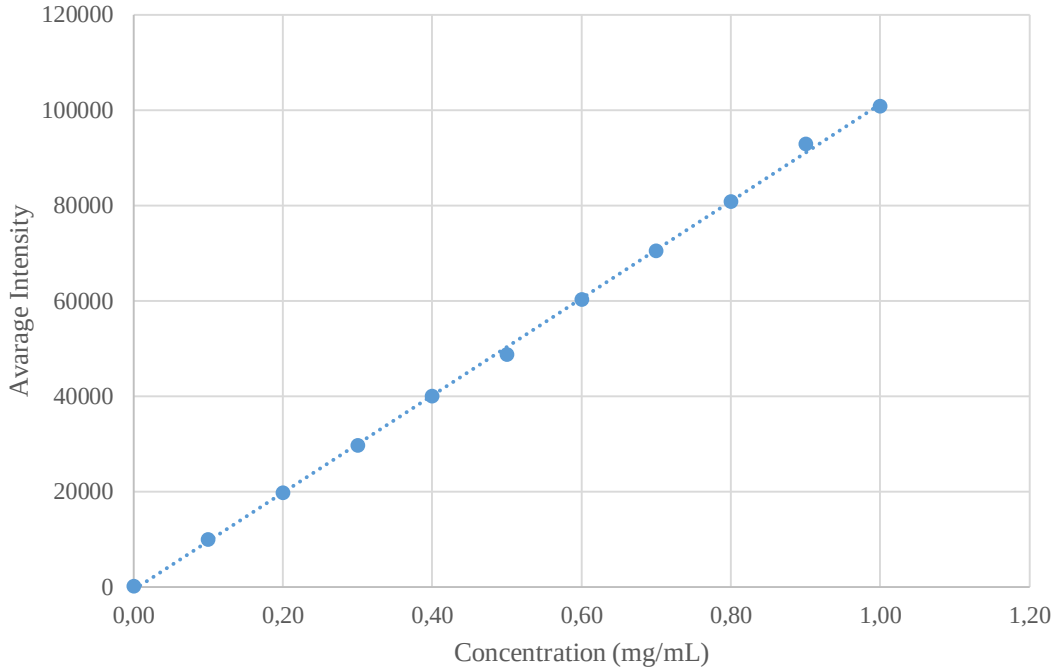
- A 0.05 molar (PBS) solution is prepared.
- Standard BSA solutions are prepared in different concentrations;

Concentration (mg/mL)	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
mL BSA (1.0 mg/mL)	0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5
mL Buffer 0.05 M PBS	5	4.5	4	3.5	3	2.5	2	1.5	1	0.5	0

- Fluorescence intensities of 11 standard solutions are measured in three repetitions with 280 nm.

Method

- The concentration is plotted against the mfluorescence intensity of the solutions.
- The fluorescence intensity of the solution of unknown concentration is measured and its concentration is calculated from this calibration line created by reading the maximum fluorescence intensity.



$Y = X.a + b$

Video

https://www.youtube.com/watch?v=9MQPp0cwI8g

Animation suggestion: https://www.youtube.com/watch?v=CcN8NnGGPhs

Data

Concentration (mg/mL)	I_1	I_2	I_3	I_{ort}
0,00	120	119	97	?
0,10	9917	9824	10015	?
0,20	19668	19891	19834	?
0,30	29481	30017	29732	?
0,40	40181	40238	39728	?
0,50	48671	48915	48794	?
0,60	59903	60740	60342	?
0,70	70476	70676	70305	?
0,80	81075	80699	80735	?
0,90	96961	90833	90749	?
1,00	100969	100672	100750	?

Unknown Sample Fluorescence Intensity

44592

Final Report

- How do you verify your result? (Literature or additional method?)
- When the BSA structure is examined in detail, how is the photoluminescence property achieved.
- What are the considerations in the experimental setup?
- What are the other spectroscopy methods? What are the advantages and disadvantages?
- Discuss the applicability of fluorescence spectroscopy in HSA (Human Serum Albumin) analysis.

- Submission date:

Dec 25 2020

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