Fluorescence Techniques

Dr Manaf Abdulrahman Guma
University Of Anbar- College Of Applied Sciences-Hit(Heet)
Department Of Applied Chemistry

1

What we do study in the biological system?

- We study proteins-protein interactions.
- Proteins are the biomolecules which are units to build the body.
- Proteins as molecules have dynamics.

2

What is fluorescence

- Fluorescence is a phenomenon of the molecule adsorbs of light energy at one wavelength and re-emit it at another, usually longer, wavelength with low energy.
- Some molecules fluoresce naturally.
- Others can be modified to make it fluoresce.
- Fluorescence compounds have two characteristic spectra:

1. An excitation spectrum: wavelength and amount of light absorbed.
2. An emission spectrum: wavelength and amount of light emitted.

- The spectra are the signature or fingerprint of the compounds.
- There is No two compounds have the same fluorescence signature.

3

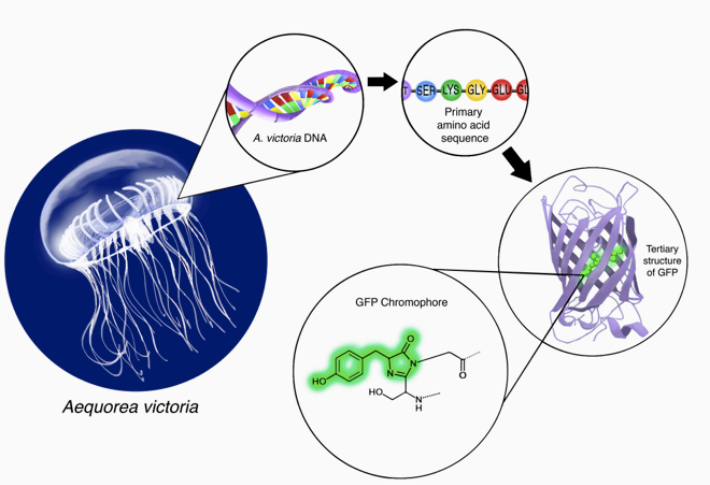
Does fluorescence occur in nature? How?

- Fluorescence occurs in nature as in jellyfish.
- This type of fishes have proteins responsible for fluorescence.
- It is called GFP.

4

Jelly Fish and Green Fluorescent Protein GFP

- What makes jelly fish fluoresce?
- A protein found in the fish which genetically involved in the sequence called GFP.
- What happed when scientists replaced the DNA code of florescence?
- No more florescence showed.



5

Biophysical Instrumentation

- What is the measurement of the fluorescence?
- Fluorometry the measurement of the fluorescence.
- What is the instrument used to measure fluorescence?
- Fluorimeter is the instrument used to measure fluorescence.
- Explain how?
- The fluorimeter generates the wavelength of the light that is required to excite the analyte of interest.
- It then transmits the wavelength of light emitted; and then measures the intensity of the emitted light.

6

What are Fluorophore and chromophore?

- **The chromophore** is (chemistry) that part **of** the molecule **of** a dye responsible **for** its colour while …
- **The fluorophore** is (biochemistry) a molecule or functional group which is capable **of** fluorescence.
- So, not every **chromophore is fluorophore but ..**
- **Each fluorophore is chromophore.**

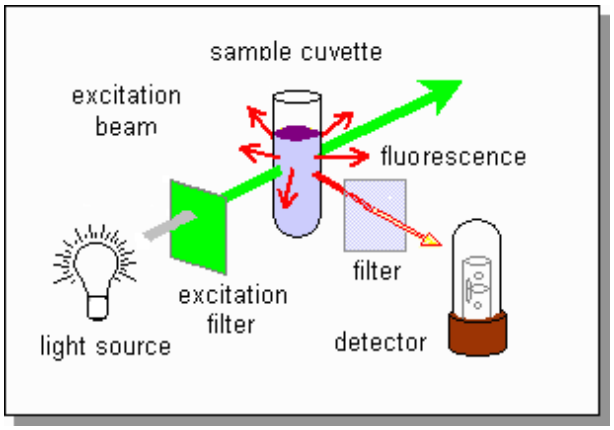
7

Describe the Fluorescence mechanism?

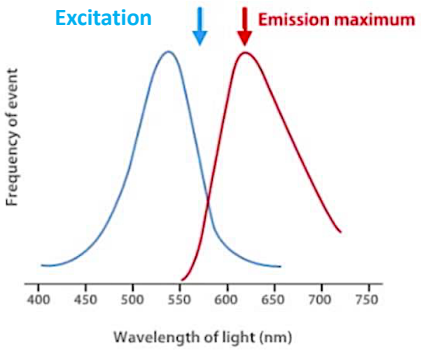
- When a photon of excitation light is absorbed by an electron of a fluorescent particle called fluorophores or simply Flours (low energy), which increases the energy level of the electron to an excited state.
- The energy is emitted as a photon to bring the electron back to its ground state (in a single step).
- This emission show fluorescence.

8

Describe Fluorescence basic?



draw spectra shape



Fluorimeters employ monochromators (a spectrofluorometric) or optical filters (a filter fluorimeter).

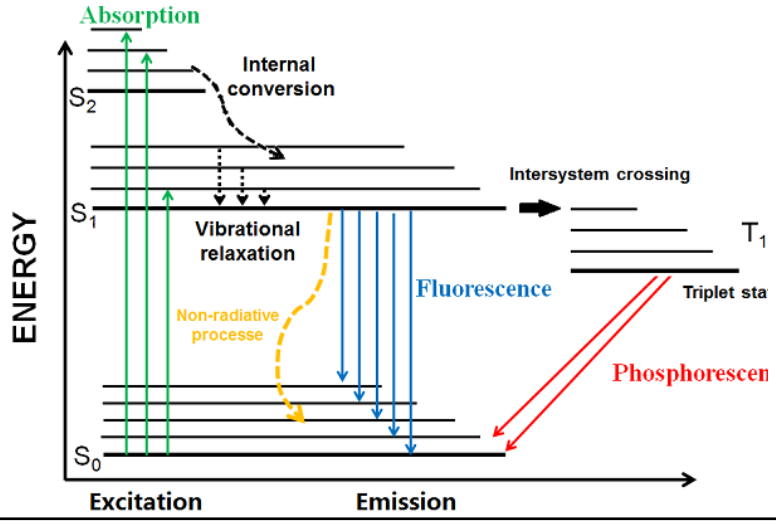
9

Which has longer wavelength?

- Why the emitted fluorescence can be distinguished from the excitation light?
- The emitted photon usually carries less energy and therefore has a longer wavelength than the excitation photon.

10

Describe Fluorescence mechanism? Jablonski diagram



11

Jablonski diagram: 3-stages process:

- **So, Fluorescence is the results of 3-stages process:**
- Excitation of fluorophore due to the absorption of light energy.
- Transient of light exciting time with loss of some energy (very short 10^{-9} -10^{-15} sec).
- Return the fluorophore to the ground state with an emission of light.

12

What are the experimentational artifacts of Florescence?

- Photo-bleaching:
- It occurs when a fluorophore permanently loses the ability to fluoresce due to photo-induced chemical damage or modification.
- Quenching:
- Process leads to reduce the fluorescence intensity or the quantum yield.
- Wrong concentrations

13

How can we use fluorescence in biology

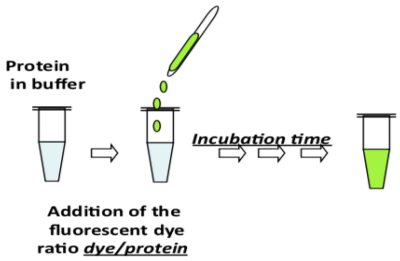
- There too many applications for the fluorescence in biology: e.g

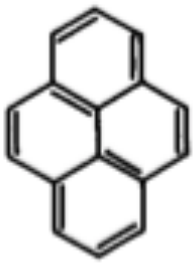
1. DNA & RNA sequencing.
2. To measure the conformational change upon the protein-protein interactions.
3. Enzymatic assays
4. Microscopy
5. **Cool fluorescent(biosensors.)** e.g: Food contamination etc.
6. **Diagnostic in medicine: Fluorescence imaging in cancer detections.**

14

How do we use florescence to study protein?

- Fluorescence is used for protein-protein interactions.
- Protein-protein interactions could be measured by following the conformational change due to the binding.
- We need to label the protein by a fluorophore (a dye)



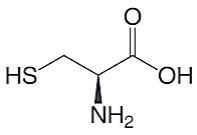


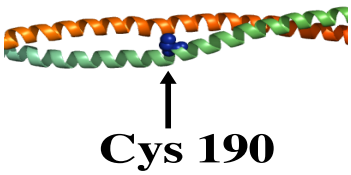
Pyrene

15

How can we label the protein by fluorophore dye?

- Find the site that dye can attach to it.
- example: a side chain of cysteine in the protein has -SH group.
- But it is found S-S in the protein?
- Unfold the protein by urea.
- React the dye with -SH= S+dye.
- Refold the protein.

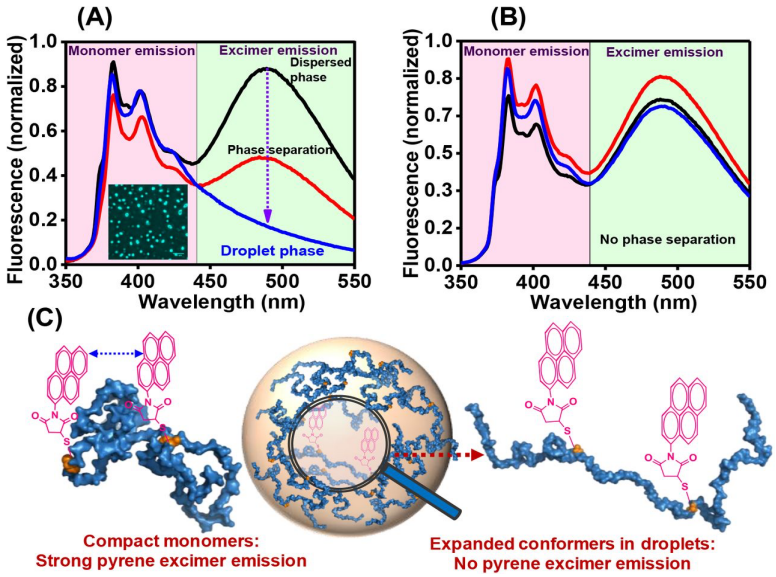




Cys 190

16

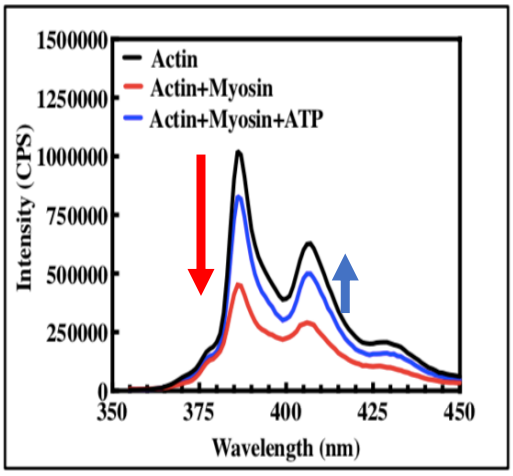
For explanation only.



17

Then we do an assay to detect the binding

- The change in the spectra is upon adding another protein.
- The change in the spectra is due a conformational change in the structure.

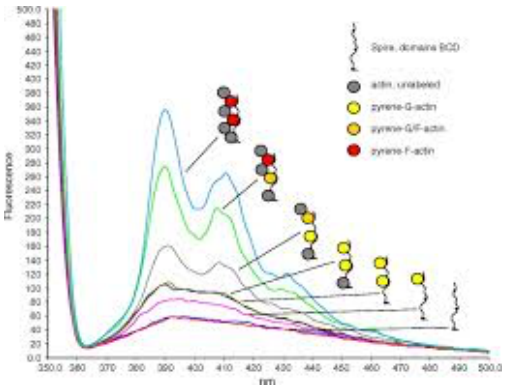


4 The emission spectrum of PIA-actin alone and upon addition of myosin heads and ATP.

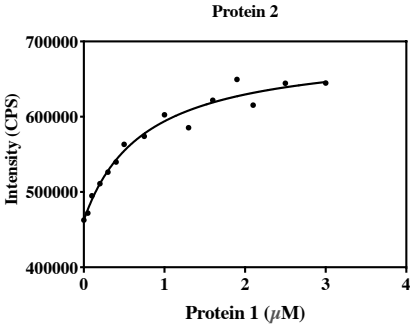
18

How can we do protein- protein titration?

1. By following measuring the change of florescence due to adding another protein.
2. The change result form the conformation change in the structure of the protein.



Then we fit the points to calculate Kd (dissociation constant)

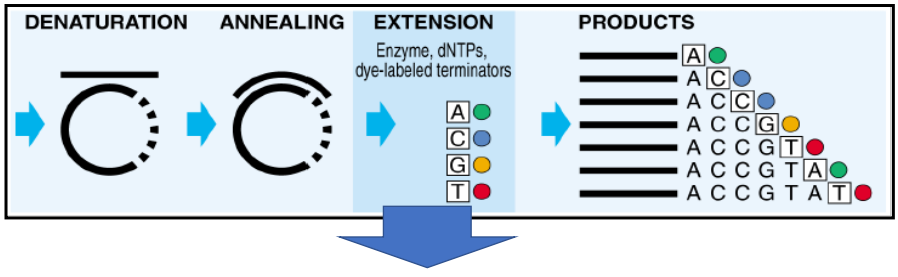


$$y = \frac{Vmax * x}{Kd + X}$$

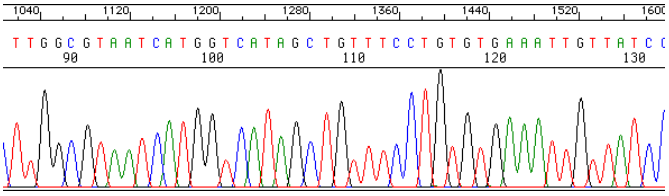
19

How do we use Fluorescence for DNA & RNA sequencing?

Labelling the Terminator Sequencing of each nucleotide with a fluorophore dye



Then we check the sequence of DNA by software…



20