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2. Fluorescent Molecules and Dyes

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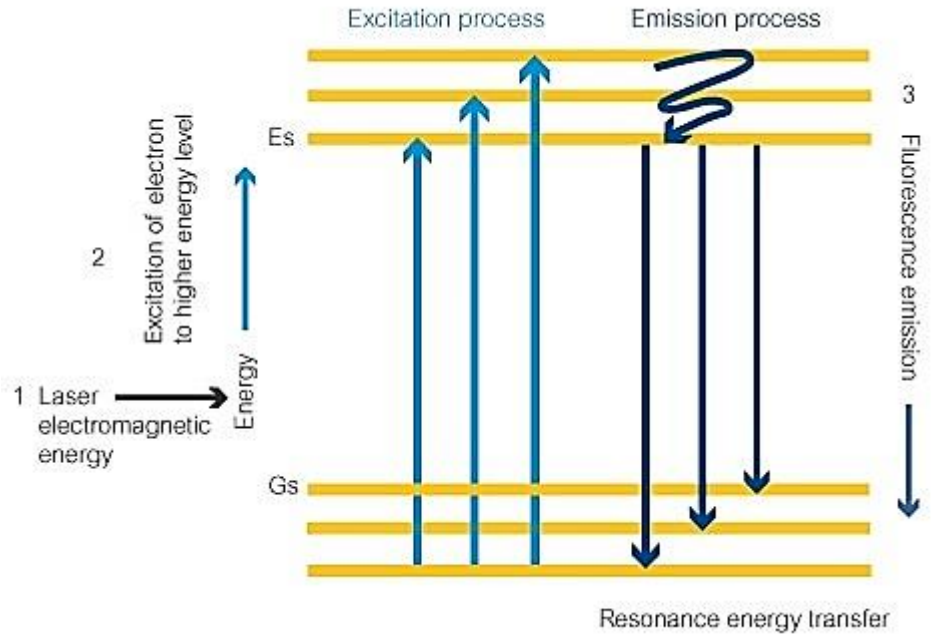
2.1 Introduction:

Fluorescent compounds, commonly known as fluorophores react to light differently from ordinary molecules. Fluorophores can emit a large number of photons as a result of this excitation and emission cycle, and fluorescent compounds are used in a wide range of research applications. The fluorescent moiety comprises electrons that can absorb a photon and temporarily enter an excited state before either dispersing the energy non-radiatively or emitting it as a photon, even if with lower energy, i.e., at a longer wavelength. Fluorescence, emission of electromagnetic radiation, usually visible light, caused by excitation of atoms in a material, which then reemit almost immediately (within about 10^{-8} seconds). For decades, scientists have utilized fluorescent dyes, often known as reactive dyes or fluorophores. Compared to fluorescent proteins, fluorescent dyes have better photostability and brightness, and they don't require any maturation time. A fluorophore (also known as a fluorochrome) is a fluorescent chemical substance that may re-emit light after being excited by light. Fluorophores are often made up of a combination of aromatic groups, or planar or cyclic compounds with many linkages.



The correct quantity of energy is provided to an electron in a fluorescent dye molecule by electromagnetic energy from a laser set at the correct wavelength. This wavelength is the molecule's characteristic excitation. Energy is absorbed by this electron. The electron advances to the next energy level after absorbing this energy and into an excitation state (Es).

Finally, the energy is released as a photon (fluorescence), and the electron returns to a lower energy level. Fluorescence is based on the property of some molecules that when they are hit by a photon, they can absorb the energy of that photon to get into an excited state. Upon relaxation from that excited state, the same molecule releases a photon: fluorescence emission. The quantity of energy released is determined by how far the electron descends the energy levels, which in the same fluorescent molecule will always be the same. The fluorescent dye's signature emission wavelength is determined by the wavelength of the photon and the color of the fluorescence detected.

The fluorophore absorbs light energy of a particular wavelength and re-emits light of a different wavelength. As the molecule in its excited state interacts with surrounding molecules, the absorbed wavelengths, energy transfer efficiency, and duration before emission are all affected by the fluorophore structure and its chemical environment. The common phrases used to refer to a specific fluorophore are the wavelengths of maximal absorption (excitation) and emission (for example, Absorption/Emission = 485 nm/517 nm), however, the entire spectrum may be relevant to consider. The excitation wavelength spectrum might be very narrow or very broad, or it can go all the way beyond a cut-off point. The emission spectrum is frequently sharper than the excitation spectrum, with a longer wavelength and lower energy as a result. Excitation energies range from ultraviolet to visible light, with emission energies extending from visible light into the near-infrared. Fluorescence is widely employed for its basic qualities, such as a marker of labeled components in cells (fluorescence microscopy) or as a solution indication (fluorescence spectroscopy), but it has additional properties not present in radioactivity that make it even more widely used.

2.2 Common Fluorescent Labels:

Fluorescent reporter labels (fluorochromes) are chemical compounds that absorb light at one wavelength and then re-emit it at a different wavelength. Because the molecule loses energy to its environment before fluorescing, light is emitted at longer wavelengths. Internal conversion is the term for this procedure. Electrons in a fluorescent molecule can be in a ground state (S0), or they can be excited to higher levels by the light of a specified wavelength (S1, S2). Following excitation, electrons return from S2 to S1, effectively completing the internal conversion process. The phenomenon of fluorescence is caused by further relaxation from this state to S0, which is accompanied by the emission of a photon.

The peak in the excitation and emission spectra corresponds to the maximum excitation and emission wavelength. The molar absorption coefficient is a relationship between the amount of absorbed light and the concentration of fluorophore in solution at a specific wavelength. Quantum yield (= number of emitted photons per absorbed photons) is the efficiency with which energy is transferred from incident light to emitted fluorescence.

The duration of a fluorophore's excited state before it returns to its ground state (in picoseconds). It is the time it takes for an excited fluorophore population to decline to 1/e (0.368) of its original amount. Stokes shift is the difference in wavelengths of maximum excitation and maximum emission.

The Stokes shift is the difference between the excitation and emission wavelengths, while a lifespan is the time it takes an excited electron to produce a photon. The extinction coefficient is the quantity of light that can be absorbed by a fluorophore, and the quantum yield is an indicator of the dye's efficiency. The quantum yield and extinction coefficient are unique to each fluorophore, and when multiplied together, the fluorescent molecule's brightness is calculated.

Following light excitation, a fluorescent chemical emits light. Non-covalently binding fluorochromes and covalently binding fluorochromes are the two primary families of fluorochromes.

The dark fraction refers to the percentage of molecules that are active in fluorescence emission. Long-term single-molecule imaging of quantum dots revealed that 20-90 percent of the particles never generate fluorescence. Conjugated polymer nanoparticles, on the other hand, have essentially no black component in their fluorescence. Protein misfolding or poor chromophore synthesis can cause a dark fraction in fluorescent proteins.

Other features, such as photobleaching and photo resistance, are influenced by these attributes. Other elements that can affect fluorophore behavior include the polarity of the fluorophore molecule, fluorophore size and shape, and others. Fluorophores include particles with a diameter of 2-10 nm and a mass of 100-100,000 atoms, such as quantum dots.

The fluorophore's size may sterically obstruct the tagged molecule, affecting the fluorescence polarity. Fluorophore molecules could be used alone or as part of a functional system's fluorescent theme. Fluorophore molecules can be divided into four categories based on molecular complexity and synthetic methods: proteins and peptides, tiny chemical compounds, synthetic oligomers and polymers, and multi-component systems.

Fluorescent dyes have the unusual property of absorbing UV light while releasing light in the visible spectrum. Non-destructive testing, water tracing, leak detection, antifreeze, adhesives, car wash soaps, and detergents are just a few of the applications for fluorescent dyes.

What are Tandem Dyes and How Do They Work?

Tandem fluorescent dyes are 'dual' fluorescent molecules that have been conjugated. PE-Cy5 is one such example. They will be close enough to the antibody for energy to be passed between them. The donor molecule (e.g., PE) will be excited by the laser excitation wavelength, but it will not be the right wavelength for the acceptor molecule.

The energy supplied by the donor molecule will excite the electron in the acceptor molecule at the precise wavelength. The energy is subsequently released by the acceptor molecule in the form of a photon with its unique wavelength.

An electron in the donor fluorescent dye molecule will get the ideal quantity of energy from electromagnetic energy from a laser set at the right wavelength. This is the molecule's characteristic excitation wavelength. The energy is absorbed by this electron. The electron takes the energy and moves to the next energy level, Es, where it becomes excited.

The absorbed energy is released as a photon by the electron. The electron returns to its lower energy state as a result of this. A photon is released as the energy is released. This causes an electron in the tandem dye molecule to be excited, causing it to move up to the next energy level.

Finally, the electron's energy is released in the form of a photon (fluorescence), and it returns to a lower energy level. The amount of energy released is proportional to the number of energy levels passed by the electron. In the same fluorescent molecule, this will always be the same. The wavelength of the photon and the color of the emitted fluorescence are thus determined by the energy emitted. Minerals and gemstones often emit visible colors when UV rays fall on them. Diamond, rubies, emeralds, calcite, amber, etc. show the same phenomenon when UV rays or X-rays fall on them. One of the best fluorescence examples in nature is Bioluminescence and Chemiluminescence.

2.3 Bioluminescence and Chemiluminescence:

Fluorescence, chemiluminescence, and phosphorescence are three forms of luminescence qualities, which refer to a substance's ability to emit light. Fluorescence is a trait in which light is absorbed and emitted at a lower energy in a few nanoseconds (about 10ns), whereas bioluminescence is biological chemiluminescence, a property in which light is generated by an enzyme's chemical reaction on a substrate.

Phosphorescence is a feature of materials that allows them to absorb light and then emit it several milliseconds later or more (due to forbidden transitions to the ground state of a triplet state, while fluorescence occurs in excited singlet states). Because of the size of the inorganic particles, they could not be used in life science study until recently. However, because transition metal-ligand complexes, which combine metal and numerous organic moieties, have extended lifetimes, up to several microseconds, the distinction between fluorescence and phosphorescence is not clear.

2.4 Fluorescent Dyes:

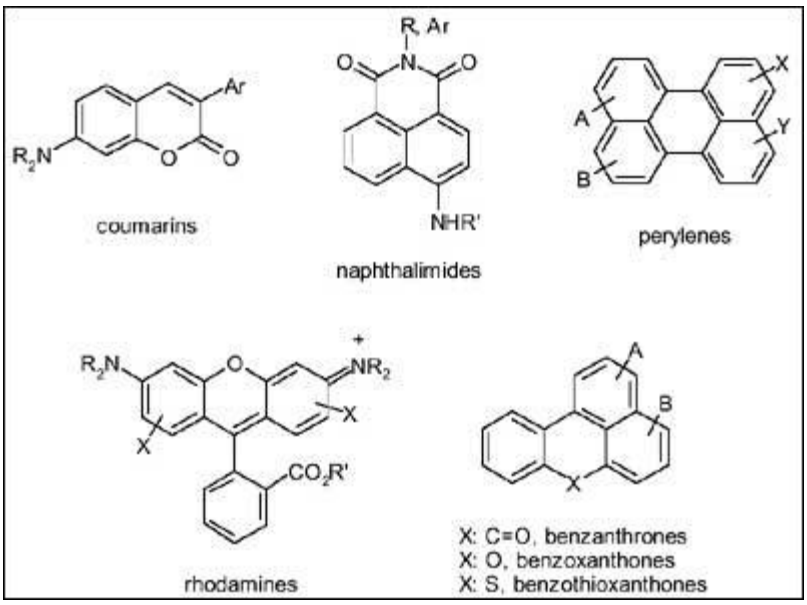
Dyes that glow in the dark:

For decades, scientists have utilized fluorescent dyes, often known as reactive dyes or fluorophores. Compared to fluorescent proteins, fluorescent dyes have better photostability and brightness, and they don't require any maturation time. Antibody conjugates or peptide tags, on the other hand, are frequently used to target fluorescent dyes to proteins of interest.

This necessitates cell fixation, which makes genetic circuit dynamics measurement unfeasible. Although several fluorescent dyes may be utilized in living cells, their application is still limited in many circumstances.

2.4.1 Fluorescent Dyes Types:

Fluorescent dyes come in a wide variety of colors. The following are some of them, along with their chemical structures:



Fluorescent Dyes' Distinctive and Productive Features Fluorescent Dyes are known for their unique and productive features, which include:

- Extremely detectable.
- Solubility in water.
- Toxicity levels are extremely low.
- In a normal aquatic environment, there is a fair amount of stability.
- Fastness qualities are excellent.
- Colour intensity is high.

Non-protein organic fluorophores belong to the following major chemical families:

- Xanthene derivatives: fluorescein, rhodamine, Oregon green, eosin, and Texas red
- Cyanine derivatives: cyanine, indocarbocyanine, oxacarbocyanine, thiacarbocyanine, and merocyanine
- Naphthalene derivatives (dansyl and Prodan derivatives)
- Coumarin derivatives
- Oxadiazole derivatives: pyridyloxazole, nitrobenzoxadiazole
- Anthracene derivatives: anthraquinones,
- Pyrene derivatives: cascade blue, etc.
- Oxazine derivatives: Nile red, Nile blue, cresyl violet, oxazine 170, etc.
- Acridine derivatives: proflavin, acridine orange, acridine yellow, etc.
- Arylmethine derivatives: auramine, crystal violet, malachite green
- Tetrapyrrole derivatives: porphin, phthalocyanine, bilirubin

2.4.2 Fluorophores Have The Following Main Characteristics:

Delocalized electrons glow in these fluorophores, allowing them to leap a band and stabilize the energy absorbed. For example, benzene, one of the most basic aromatic compounds, is stimulated at 254 nm and emits at 300 nm. Quantum dots, which are fluorescent semiconductor nanoparticles, are distinguished from fluorophores by this property.

Delocalized electrons in organic fluorophores can jump a band and stabilize the energy absorbed, which is why most fluorophores are conjugated systems. There are several families of exits, with excitations ranging from infrared to ultraviolet.

Lanthanides (chelated) are fluorescent metals that emit due to forbidden transitions involving 4f orbits, resulting in low absorption coefficients and sluggish emissions, necessitating stimulation via fluorescent organic chelators. Transition metal-ligand complexes, which produce molecular fluorescence from a partially prohibited metal-to-ligand charge transfer state, are the third class of small-molecule fluorophores. These are often complexes of Ruthenium, Rhenium, or osmium.

2.4.3 Fluorescence in The Life Sciences:

Fluorescence is commonly utilized in the life sciences as a non-destructive method of tracking and analyzing biological components. Intrinsic fluorescence or autofluorescence refers to the fact that some proteins or tiny molecules in cells are innately luminous. Alternatively, an extrinsic fluorophore, a fluorescent dye that can be a tiny molecule, protein, or quantum dot, can be used to "label" specific or general proteins, nucleic acids, lipids, or small molecules. Several techniques exist to exploit additional properties of fluorophores, such as fluorescence resonance energy transfer, in which energy is transferred non-radiatively to a specific neighboring dye, allowing proximity or protein activation to be detected; another is the change in properties, such as intensity, of certain dyes depending on their environment, allowing their use in structural studies.

2.4.4 Fluorescent Proteins:

Green Fluorescent Protein (GFP) from the jellyfish Aequorea victoria, which spontaneously fluoresces upon folding via certain serine-tyrosine-glycine residues, is the most important one as a research tool. GFP and other fluorescent proteins have the advantage over organic dyes or quantum dots in that they can be expressed exogenously in cells or as a fusion protein, which is a protein made by ligating a fluorescent gene to another gene and whose expression is controlled by a housekeeping gene promoter or another specific promoter. Fluorescent proteins can be utilized as reporters for a variety of biological events, including subcellular localization and expression patterns, using this method. GFP is found naturally in corals, particularly the Anthozoa, and various mutants have been developed to span the visible spectra and fluoresce longer and more consistently. Other fluorescent proteins require a fluorophore cofactor and can thus only be employed in vitro; these are frequently found in plants and algae.

2.4.5 Fluorescence Lifetime:

Conjugated dyes have a lifetime of 1–10 ns, with a few exceptions, such as pyrene, which has a lifetime of 400ns in degassed solvents or 100ns in lipids, and coronene, which has a lifetime of 200ns. The fluorescent organometals (lanthanides and transition metal-ligand complexes) are a separate group of fluorophores that have substantially longer lifetimes due to the limited states: lanthanides have lifetimes of 0.5 to 3 ms, while transition metal-ligand complexes have lifetimes of 10 ns to 10 µs. The lifetime of a fluorescent dye should not be confused with the lifetime of photo destruction of the "shelf-life" of a dye.

2.4.6 Fluorescent Dyes for Textiles:

The Colour Index does not recognize fluorescent dyes as a distinct, defined dye application class. Rather, the few commercially important textile fluorescent dyes are scattered among the typical application classes. The use of rhodamine and similar chemicals to dye silk with extraordinarily bright colors was the first commercial application of fluorescent dyes. The Colour Index only lists a small number of fluorescent dyes that have been identified as acceptable for use with natural protein and cellulose fibers.

It was billed as the first fluorescent reactive dye for cellulosic fibers that could be used in high-visibility workwear and athletics. It's said to be compatible with other Remazol reactive dyes, and it's particularly useful for dyeing dazzling yellow tints on cellulosic polyester polyamide.

Fluorescent dyes are far more important for use with synthetic fibers including polyester, polyamides, and polyacrylonitrile, as well as elastane fibers. The most important textile applications are polyester; hence dispersion dyes are the most important fluorescent textile dyes. Fluorescent dispersion dyes are also utilized on polyamides and cellulose acetates but to a lesser extent. The majority of commercial dyes are yellow, resulting in the well-known greenish-yellow fluorescent colors found on garments worn for safety reasons. The majority of commercial dyes are yellow, resulting in the well-known greenish-yellow fluorescent colors found on garments worn for safety reasons. There are a variety of fluorescent cationic dyes that are water-soluble. The coumarin C.I. Basic Yellow 40 and the methine dyes C.I. Basic Red 13 and C.I.

Basic Violetare two commercially important water-soluble fluorescent cationic dyes for use on acrylic fibres. Technical performance features of fluorescent dispersion dye on polyester and cationic dyes on acrylic fibers are typical of the dye application class. Commercial fluorescent dyes for textiles are frequently chastised for having lightfastness qualities that fall short of the requirements of more demanding applications. However, through the appropriate application of light stabilizing additives like UV absorbers, this property can be improved.

2.5 Fluorescent Dyes and Brighteners:

Fluorescent dyes are distinguished from fluorescent brighteners, which emit visible light but absorb ultraviolet radiation, by the fact that they absorb and emit significantly in the visible portion of the spectrum. Coumarins, the majority of which are 3,7-disubstituted, are one of the most commercially important classes of fluorescent dyes and brighteners. The presence of electron-releasing substituents at the 7-position and electron-withdrawing (typically heterocyclic) substituents at the 3-position distinguishes the colors. Red dyes (R = alkyl, X = NH, O, or S) and greenish-yellow dyes, for example, are both used to color polyester. Coumarin fluorescent whiteners have fluorescence maxima at 430–440 nm, which counteract the short-wavelength visible light absorption of fibers, paper, and plastics. Compounds with nitrogen-containing substituents at both the 3- and 7-positions have replaced the hydroxycoumarins formerly employed. In nature, they are frequently heterocyclic. There are non-ionic and water-soluble cationic coumarins for whitening polyester and polyacrylonitrile, respectively.

2.6 Fluorescent Dye Applications:

Fluorescent dyes are widely used in a variety of applications. It's utilized in crack detection, plastics, synthetic resins, printing inks, non-destructive testing (NDT), and sports ball dyeing as a penetrant liquid. Memantine Brilliant Yellow 8G is a fluorescent dye that is particularly effective for textile applications.

Fluorophores are occasionally utilized on their own as a fluid tracer, a dye for coloring certain structures, an enzyme-substrate, or a probe or indicator. They are covalently attached to a macromolecule and serve as a marker for affine or bioactive reagents in general (antibodies, peptides, nucleic acids). Fluorophores are commonly employed in a range of analytical procedures, such as fluorescence imaging and spectroscopy, to stain tissues, organisms, or materials.

One of the most widely used fluorophores is fluorescein isothiocyanate (FITC), an amine-reactive isothiocyanate derivative. Other frequent fluorophores include rhodamine derivatives coumarin and cyanine. Newer generations of fluorophores, many of which are proprietary, often outperform classic dyes with comparable excitation and emission because they are more photostable, brighter, and/or less pH-sensitive.

GFP (green), YFP (yellow), and RFP (red) fluorescent proteins can be linked to other proteins to produce fusion proteins, which are synthesized in cells after transfection with an appropriate plasmid carrier.

2.6.1 Direct Cell Count Using Fluorescent Dyes:

For bacterial counting, a variety of fluorescent dyes can be utilized. The most extensively used fluorescent dye for measuring the quantity of both living and dead bacterial cells is acridine orange. When exposed to near-ultraviolet light, the stained cells fluoresce orange, and the method is particularly useful for measuring the total number of microorganisms in samples such as soil and water, where metabolically different populations coexist.

2.6.2 Fluorescent Silica Nanoparticles:

Rapid photobleaching and low photostability are problems with organic fluorescent dyes used in biological labeling. Incorporating numerous organic fluorophores into metal oxide-based scaffolds or hybrid siloxane-oxide networks, which effectively protect the fluorophore dyes from ambient oxygen and so provide better photostability, is one way to overcome these challenges. The advantages of silica over other host matrices are its biocompatibility, optical transparency, and ease of doping with functional organic moieties. Furthermore, when compared to single-molecule fluorophores, dye-containing fluorescent silica nanoparticles have a huge number of dye molecules inside the silica matrix, resulting in much-increased brightness. Encapsulating fluorophores in silica matrices also enables surface modification of the shell to improve their hydrophilic nature and cell absorption. As a result, silica is frequently employed to create hybrid organic-inorganic materials for biological and medicinal purposes.

2.6.3 Fluorescent Dyes:

Fluorescent dyes (also known as fluorophores/reactive dyes) are essentially non-protein molecules that achieve their role in microscopy by absorbing light at one wavelength and re-emitting it at a longer wavelength. This results in fluorescence of various hues, which may be seen and evaluated.

As a result, they're frequently utilized in fluorescent labeling of various biomolecules (antibodies, peptides, proteins, and so on) for activities like medication delivery monitoring and imaging, among others. While fluorescent amino acids (tryptophan, tyrosine, and phenylalanine) are present in proteins (e.g., green fluorescent protein), they offer better photostability and brightness than fluorescent dyes. They require more maturation time than fluorescent dyes, making them less desirable.

2.6.4 Organic Fluorescent Dyes:

Organic dyes are distinguished by emissions that result from optical transitions that are delocalized across the whole chromophore or from intermolecular charge transfer transitions.

Resonant dyes emit light from an optical transition that is delocalized throughout the entire chromophore, whereas CT dyes emit light from an optical transition that is localized across the entire chromophore (Charge transfer dyes). Some of the most common resonant dyes, such as cyanines, rhodamines, and fluoresceins, have narrow absorption and emission bands that tend to mirror each other, as well as a modest, slightly solvent polarity-insensitive Stokes shift.

2.7 Types of Organic Dyes:

Fluorescein, like cyanines and rhodamine dyes, is an organic dye. Fluorescein is a highly fluorescent chemical with an absorption maximum of 494nm and an emission maximum of 521nm. When coupled to antibodies, it may be recognized even in extremely minute concentrations and is utilized in microscopy.

Fluorescein derivatives include fluorescein isothiocyanate, Oregon Green, and carboxy-naphthofluorescein, to name a few. Fluorescein, like many other fluorescent dyes, is affordable and simple to use, making it one of the most widely used dyes in biological research. Fluorescein is harmless in aqueous solutions, unlike most other colors. As a result, it's one of the few dyes that can be used to track groundwater.

2.7.1 Fluorescein Isothiocyanate (FITC):

Fluorescein isothiocyanate is a derivative of fluorescein that contains an isothiocyanate reactive group, which contributes to its reactive tendency toward anime and sulfhydryl groups present in biomolecules.

Fluorescein isothiocyanate is one of the most widely used fluorochromes, with high efficiency of energy transfer from absorbed to emitted light. FITC, on the other hand, is extremely sensitive to pH fluctuations and UV bleaching. Furthermore, because FITC is a dim dye, it should be used only for strongly expressed markers wherever possible.

One of the most often used organic fluorescent dyes/probes in flow cytometry and immunofluorescence is fluorescein isothiocyanate. With fluorescein-to-fluorescein interactions resulting in energy transfer and self-quenching when concentrated, it has a maximum/peak absorbance of 495nm and an emission wavelength of 520nm.

A. The dye can be used to tag molecules for the following purposes:

- Protein detection after electrophoretic separations
- Protein and peptide microsequencing analysis
- Capillary zone electrophoresis was used to analyze molecules.
- In bio-interactions, molecule tracking, and detection
- Detection of antigens in cells and tissue sections

B. Apoptosis can be detected by tagging DNA fragments:

Fluorescein isothiocyanate is the dye of choice for many processes because of its solubility in water, which makes it easy to use for conjugate preparation. It is also brightly fluorescent (due to large extinction coefficients and high quantum yields following conjugation), making it the dye of choice for many processes. The dye is coupled with various antibodies in flow cytometry and immunofluorescence microscopy to analyze and investigate such states as IL-17 immunodeficiency and the involvement of CD63 in renal functions, among other things.

Alexa Fluor 488 has emission and excitation maxima that are substantially identical to FITC. Alexa Flour 488, on the other hand, has a higher photostability and is less sensitive to self-quenching. Furthermore, Alexa Flour 488 is pH insensitive and, on most instruments, is brighter than FITC.

Alexa Flour 488 is useful for intracellular staining because of its higher sensitivity and environmental durability. FITC and Alexa Fluor 488 cannot be utilized at the same time due to essentially identical excitation and emission properties.

Carboxyfluorescein Diacetate (CFSE): is a cell-permeable dye that is often used to detect cell division and proliferation. CFSE is stable in non-dividing cells for several days, and the dye is evenly distributed between the two daughter cells after cell division. As a result, the initial fluorescence gradually decreases in proportion to the number of divisions.

R-phycoerythrin: is a pigment found naturally in red algae that transmits light energy to chlorophyll during photosynthesis.

PE-Texas Red: is a tandem conjugate that combines the properties of PE and Texas Red. It's a mixture of two fluorochromes, similar to other tandem conjugates, in which the excited fluorochrome can transfer its fluorescence energy to the second molecule, which then fluoresces at a different, longer wavelength.

Essentially, light from the first fluorochrome (PE) activates the second (Texas Red), which then fluoresces at a maximum wavelength of 615 nm. When utilizing PE-Texas Red conjugates with PE, special caution must be given because the emission profiles of both fluorochromes have a lot of spectral overlap.

Propidium iodide (PI): is an intercalating agent that binds non-specifically to a nucleic acid with a stoichiometry of 1 dye per 4-5 bases. Because PI binds to all nucleic acids non-specifically, nucleases must be used to discriminate between DNA and RNA in the cells.

The fluorescence of PI is increased 20-30-fold once it is linked to the nucleic acid. Because PI is membrane impermeable, it is usually kept out of living cells. PI is often used to stain DNA for cell cycle studies; however, the cells must first be permeabilized before the dye can be applied.

Allophycocyanin: is a photosynthetic pigment found in blue-green algae. APC is a very bright fluorochrome with six phycocyanobilin chromophores per molecule, making it ideal for flow cytometry applications.

APC and Alexa Fluor 647 cannot be employed at the same time due to essentially equal excitation and emission properties but differing spillover characteristics.

2.7.2 Cyanine Dyes:

Polymethine dyes between nitrogen atoms (two nitrogen atoms) with a delocalized charge describe cyanines, which are resonant dyes. Cyanines have become one of the most used fluorescent dyes for tagging nucleic acids due to their minimal non-specific binding to biomolecules and strong fluorescence.

A. Cyanines are split into two major categories:

Non-sulfonated cyanines - This group includes colours such as cy3, cy3.3, cy5, cy5.5, cy7, and cy7.5. Except for hydrochlorides of amines and hydrazides, these colours are characterised by low water solubility. During biomolecule labelling, they are first dissolved in organic solvents before being added to the solution containing biomolecules.

The first numeral reflects the number of carbon atoms present between the insolence groups, while the "Cy" stands for cyanine. The.5 suffix, on the other hand, denotes benzo-fused cyanines. Fluorescent dyes are frequently employed in organic media.

B. Sulfonated cyanines:

Sulfo-Cy3, sulfo-Cy5, and sulfo-Cy7 are the members of this group. These cyanines are distinguished by a sulfo-group that aids in the dissolving of dye molecules in the aqueous phase, as the name suggests.

These cyanines are more water-soluble than non-sulfonated cyanines, therefore they don't need to be dissolved in organic solvents for labelling. Hydrophobic proteins, nanoparticles in aqueous solutions, and sensitive proteins that may be denatured by DMSO or DMF are all commonly labelled with sulfonated cyanines.

2.7.3 Rhodamine Fluorescent Dyes:

Rhodamine is a member of the xanthene family, which also includes eosin and fluorescein. Rhodamines have good photostability and a variety of photophysical properties, making them ideal for usage as laser dyes, fluorescent probes, and pigments when compared to other dyes on the market.

They're very useful for the evaluation of the surface of polymer nanoparticles, detecting polymer-bioconjugates, imaging living cells, and analysing oligonucleotide adsorption on latexes, among other things.

The many varieties of dye are distinguished by the substituents they contain (R1, R2, R3, R4, and G). These fluorescent dyes have various photophysical properties in solution as a result of their differences (e.g., varied fluorescence lifetimes and emission maxima). While rhodamine dyes with rigidized amino groups have a high quantum yield across a wide temperature range, those with two alkyl substituents at each nitrogen have an active internal conversion, with quantum yield and fluorescence lifespan varied with temperature.

Rhodamine 101 and Rhodamine B, two of the most widely used rhodamine dyes, have the following properties:

- In acidic conditions, the carboxyl group tends to be protonated.
- The dye is made into a zwitterion basic solution.
- In less polar organic solvents, zwitterionic dye degrades to a colorless lactone.

2.8 Biological Fluorophores:

Green fluorescent protein (cloned from the jellyfish Aequorea Victoria) and its derivatives (e.g., phycoerythrocyanin, phycobiliproteins, and phycoerythrin, among others) were first utilised in biological research in the 1990s and are still widely used today.

While fluorophores are useful for plasmid expression in cells, bacteria, and numerous organs, their use has certain drawbacks, including the fact that they can be time-consuming and can alter the normal biological functioning of particular cellular proteins when fused.

Furthermore, biological fluorophores have lesser photostability and sensitivity than a variety of other fluorophores.

A. Green Fluorescent Protein (GFP):

One of the most widely used biological fluorophores, green fluorescent protein, is made up of 238 amino acids, three of which are responsible for the structure that emits visible green fluorescent light. When calcium is introduced to the jellyfish, the fluorophore reacts with aequorin (a protein) to produce blue light.

Researchers can now use the gene responsible for creating a protein to investigate specific genes and proteins thanks to DNA recombinant technology. Before putting the complex into a cell, the gene is coupled with another gene (the second gene being responsible for the creation of the target protein)

If the cell emits green fluorescence, researchers will know that the cell is capable of expressing the target gene.

B. Other biological fluorophores currently in use include:

Phycoerythrin (PE) is a pigment complex made up of red proteins that belong to the phycobiliprotein family. It's found in red algae and cryptophytes, where it serves as a supplement to the chlorophyll pigments. Because it emits intense fluorescence, the fluorophore can be attached to proteins such as antibodies for antigen detection in biological sciences. The fluorophore is rarely used in fluorescence microscopy since it photo bleaches quickly. It is, nevertheless, frequently employed in flow cytometry.

Allophycocyanin (APC) is a phycobiliprotein found in red algae that, like Phycoerythrin, belongs to the phycobiliprotein family. The fluorophore's absorbance maxima are at 650nm, while fluorescence emission peaks at 66nm when excited by laser lines at 594 and 633 nm.

Fluorophores have been reported to have a sensitivity between 5 and 10 times that of fluorescein conjugate, as well as several other advantageous features such as significant Stokes shifts, high water solubility, long-wavelength emission, and quench resistance.

While allophycocyanin is not typically utilised in applications that require photostability, it is commonly used in processes like flow cytometry, ELISA, microarrays, and other high-sensitivity applications.

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