Fluorescent Dyes Encyclopedia Article

Fluorescent Dyes

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Fluorescent Dyes

The use of fluorescent dyes is the most popular tool for measuring ion properties in living cells. Calcium, magnesium, sodium, and similar species that do not naturally fluoresce can be measured indirectly by complexing them with fluorescent molecules. The use of probes, which fluoresce at one wavelength when unbound, and at a different wavelength when bound to an ion, allows the quantification of the ion level.

Fluorescence has also become popular as an alternative to radiolabeling of peptides. Whereas labeling of peptides with a radioactive compound relies on the introduction of a radiolabeled amino acid as part of the natural structure of the peptide, fluorescent tags are introduced as an additional group to the molecule.

The use of fluorescent dyes allows the detection of minute amounts of the target molecule within a mixture of many other molecules. In combination with light microscopic techniques like confocal laser microscopy, the use of fluorescent dyes allows three-dimensional image constructs to be complied, to provide precise spatial information on the target location. Finally, fluorescence can be used to gain information about phenomena such as blood flow and organelle movement in real time.

The basis of fluorescent dyes relies on the absorption of light at a specific wavelength and, in turn, the excitation of the electrons in the dye to higher energy levels. As the electrons fall back to their lower pre-excited energy levels, they re-emit light at longer wavelengths and so at lower energy levels. The lower-energy light emissions are called spectral shifts. The process can be repeated.

Proper use of a fluorescent dye requires 1) that its use does not alter the shape or function of the target cell, 2) that the dye localizes at the desired location within or on the cell, 3) that the dye maintains its specificity in the presence of competing molecules, and 4) that they operate at near visible wavelengths. Although none of the dyes in use today meets all of these criteria, fluorescent dyes are still useful for staining and observation to a considerable degree.