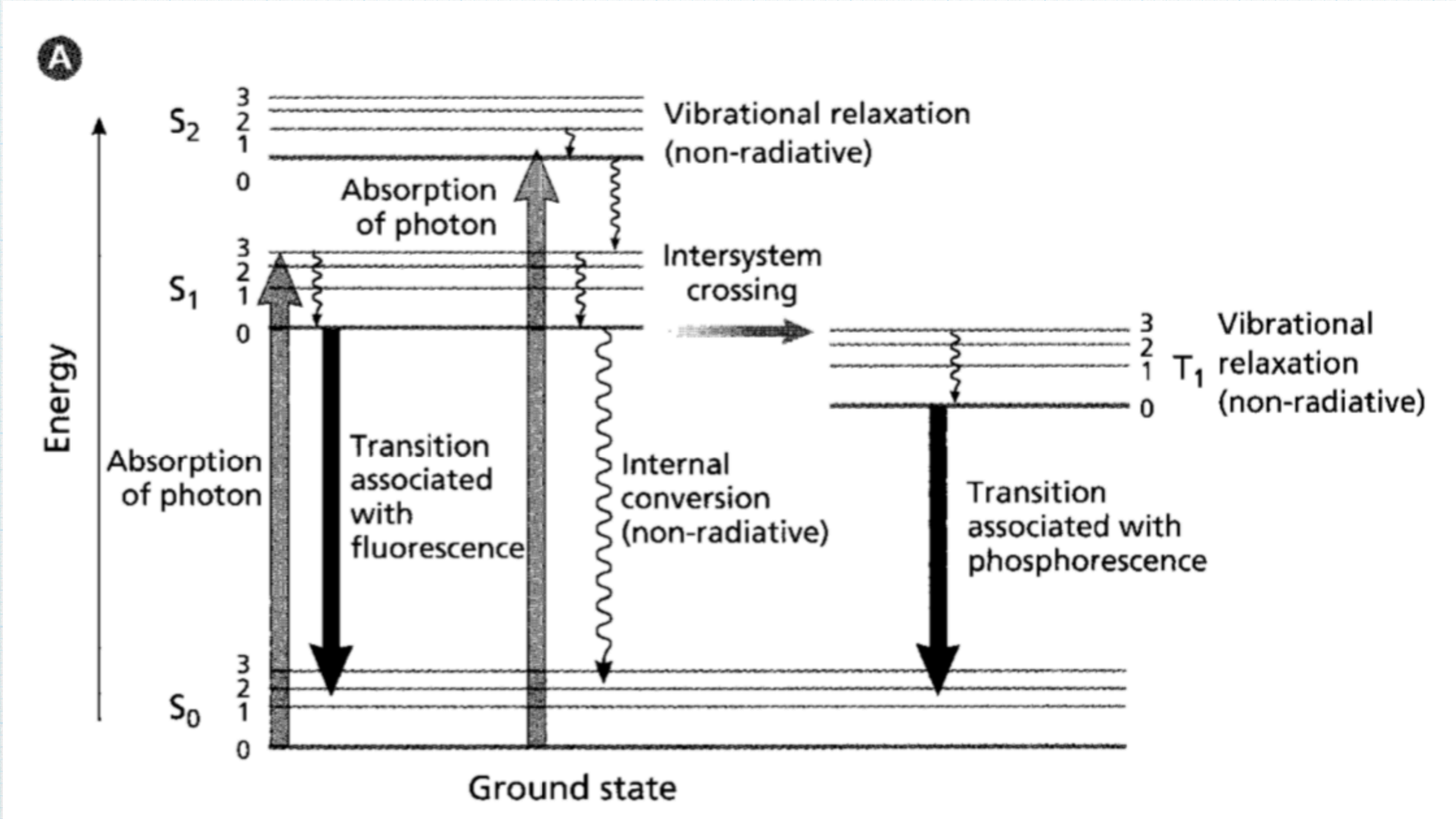
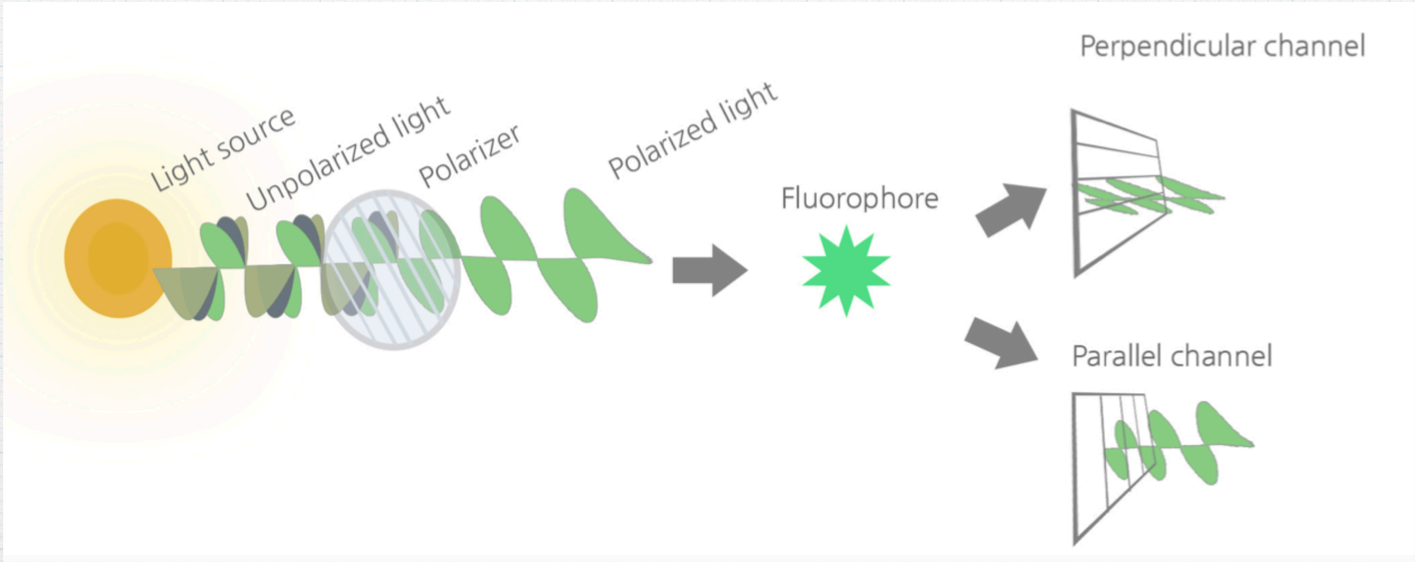
Fluorescence

A photon of excitation light is absorbed by an electron of a fluorescent particle, which raises the energy level of the electron to an excited state



Fluorescence Polarisation

Fluorescence Polarization (FP) technology measures changes in light polarization emitted by a fluorescent tracer in a sample and is quite different from fluorescence intensity, which measures the intensity of emitted light at a specific wavelength.



Fluorescence depolarisation

If the chromophore is excited with plane polarised light and the fluorescence is observed through analysing polariser, then it is found that the degree of polarisation of the fluorescence usually decreases. This phenomena is called fluorescence depolarisation.

The degree of fluorescence polarization (P) is defined as the difference between the fluorescence intensity parallel and perpendicular relative to the plane of excitation, divided by the total fluorescence intensity:

$$P = \frac{I_{II} - I_{\perp}}{I_{II} + I_{\perp}}$$

$I_{\parallel}$ = Fluorescence Intensity parallel to plane excitation

$I_{\perp}$ = Fluorescence Intensity perpendicular to pla of excitation

Fluorophore

A fluorophore is a chemical compound that is fluorescent, meaning it emits strong glowing colours. There are three key groups of chemical compounds that can fluoresce:

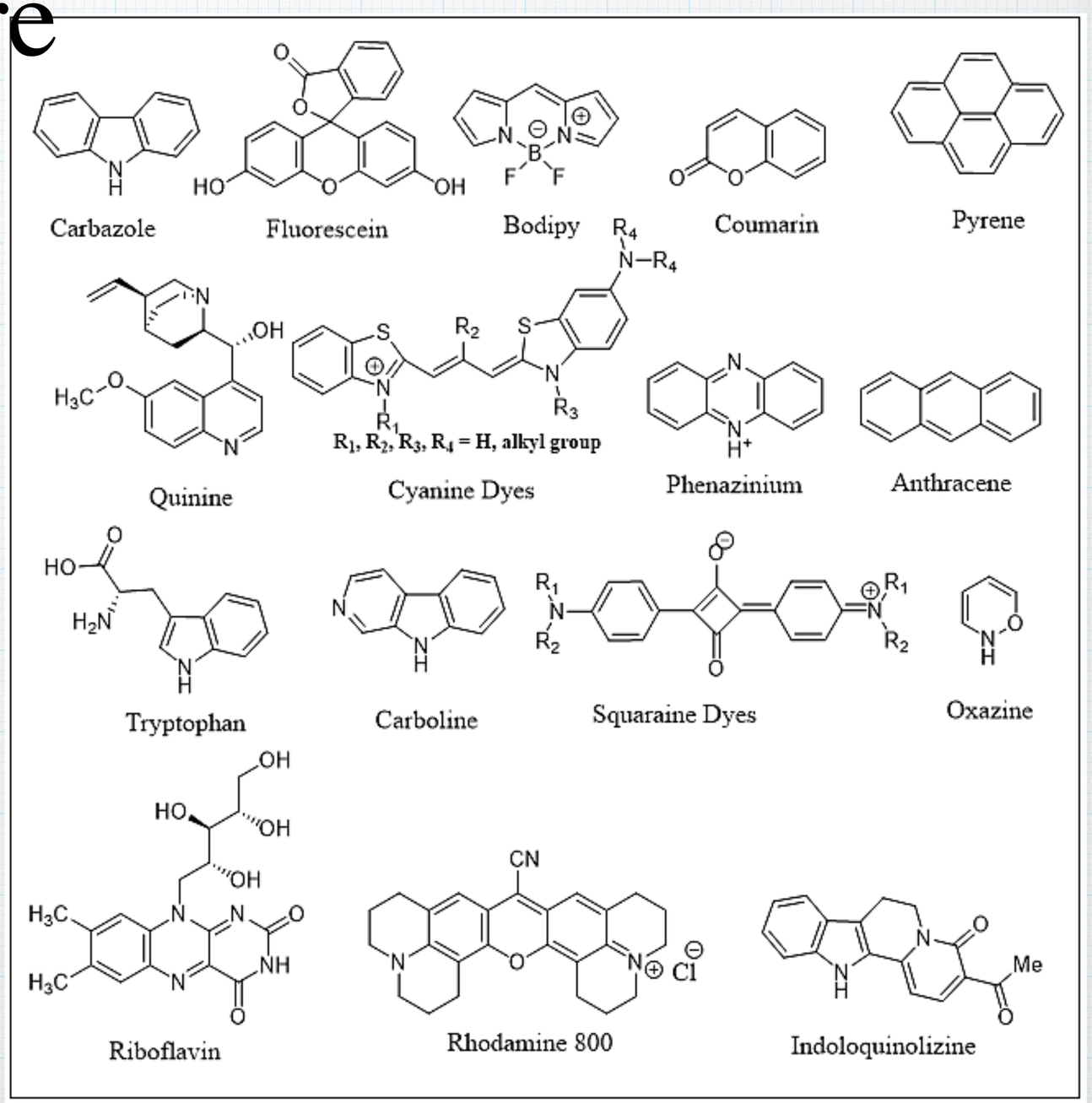
- **Quantum dots**
- Conjugated organic molecules
- Some proteins

Fluorescence in fluorophores happens when a photon is absorbed and is then re-emitted as another photon, usually of lower energy. This process happens through the transition of electrons between energy levels.

Fluorophore

fluorophore	excitation λ_{max} (nm)	emission λ_{max} (nm)
phenylalanine	258	284
tyrosine	276	302
tryptophan	280	357
vitamin A (retinol)	346	480
vitamin B_2 (riboflavin)	270 (382, 448)	518
vitamin B_6 (pyridoxin)	328	393
vitamin E (α-tocopherol)	298	326
NADH	344	465
ATP	292	388
chlorophyll *a*	428	663
hematoporphyrin	396	614

[a] Data from the FoodFluor Database at www.models.kvl.dk.



0 - 0 Transition

One transition, that from the lowest vibrational level in the ground electronic state to the lowest vibrational level in the first excited state, the 0 - 0 transition, is common to both the absorption and emission phenomena, whereas all other absorption transitions require more energy than any transition in the fluorescence emission. We can therefore expect the emission spectrum to overlap the absorption spectrum at the wavelength corresponding to the 0 - 0 transition and the rest of the emission spectrum to be of lower energy, or longer wavelength

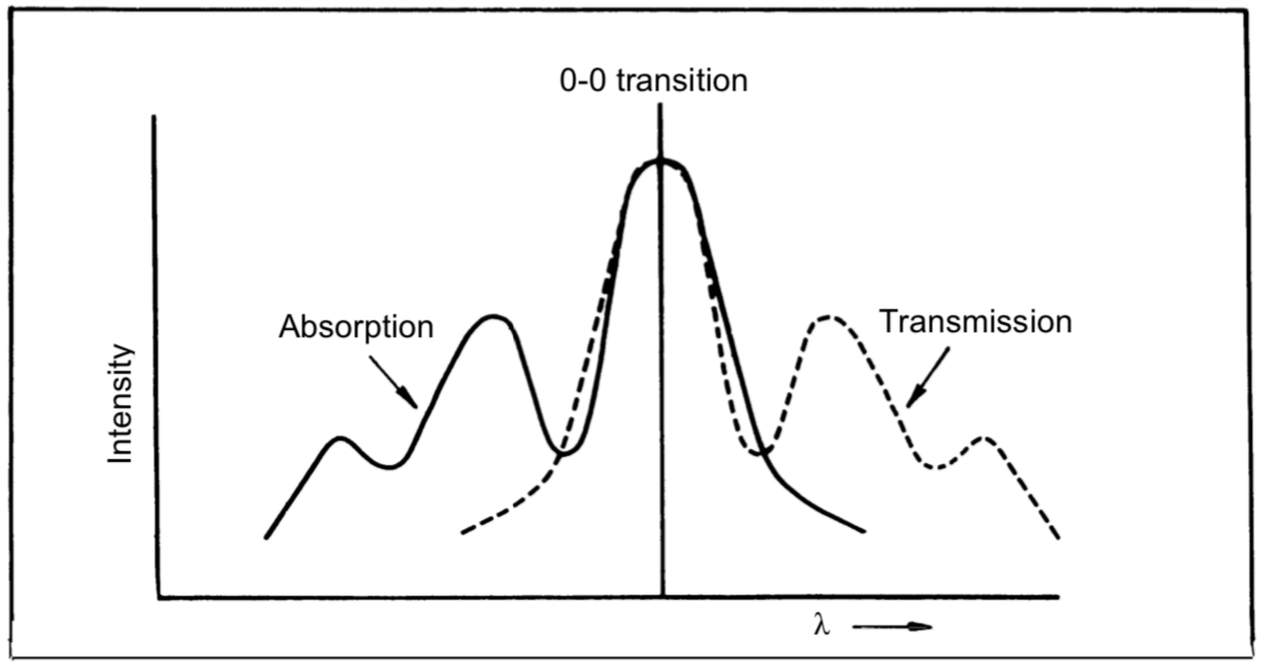


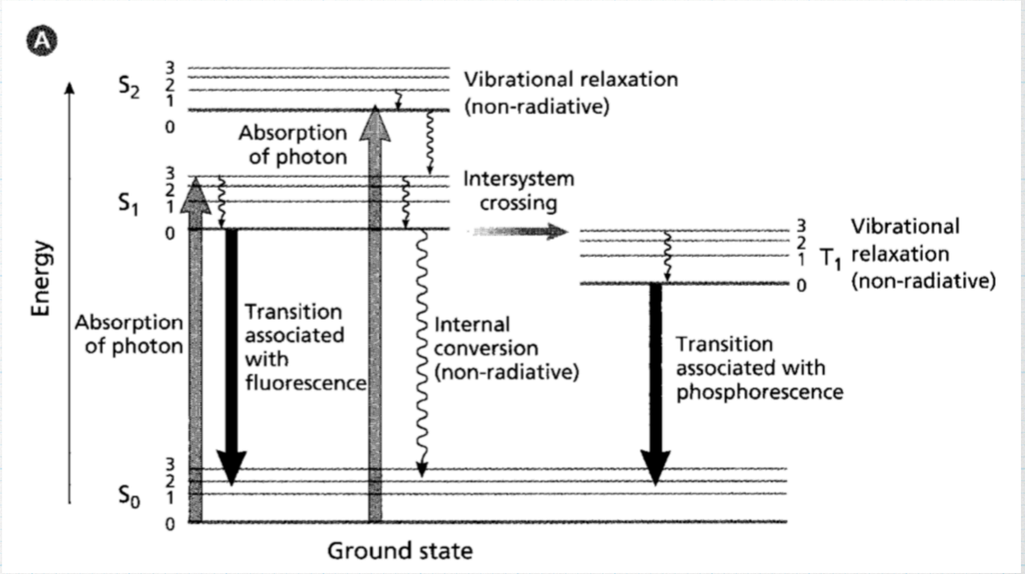
Figure 2 Idealised absorption and emission spectra

In practice, the 0-0 transitions in the absorption and emission spectra rarely coincide exactly, the difference representing a small loss of energy by interaction of the absorbing molecule with surrounding solvent molecule

Emission Spectra Are Typically Independent of the Excitation Wavelength

Kasha's rule: The same fluorescence emission spectrum is generally observed irrespective of the excitation wavelength

Upon excitation into higher electronic and vibrational levels, the excess energy is quickly dissipated, leaving the fluorophore in the lowest vibrational level of S1. This relaxation occurs in about 10–12 s, and is presumably a result of a strong overlap among numerous states of nearly equal energy. **Because of this rapid relaxation, emission spectra are usually independent of the excitation wavelength.**



The absorption of energy to produce the first excited state does not perturb the shape of the molecule greatly and this means that the distribution of vibrational levels is very similar in both the ground and first excited states. The energy differences between the bands in the emission spectrum will be similar to those in the absorption spectrum and frequently the emission spectrum will be approximate to a mirror image of the absorption spectrum. Since the emission of fluorescence always takes place from the lowest vibrational level of the first excited state, the shape of the emission spectrum is always the same, despite changing the wavelength of exciting light.

Mirror Image Rule

Following photon absorption, an excited fluorophore will quickly undergo relaxation to the lowest vibrational energy level of the excited state. An important consequence of this **rapid internal conversion** is that all subsequent relaxation pathways (fluorescence, non-radiative relaxation, intersystem crossing, etc.) proceed from the lowest vibrational level of the excited state (**S(1)**). As with absorption, the probability that an electron in the excited state will return to a particular vibrational energy level in the ground state is proportional to the overlap between the energy levels in the respective states (Figure 2). Return transitions to the ground state (**S(0)**) usually occur to a higher vibrational level (see Figure 3), which subsequently reaches thermal equilibrium (vibrational relaxation). Because emission of a photon often leaves the fluorophore in a higher vibrational ground state, the emission spectrum is typically a mirror image of the absorption spectrum resulting from the ground to first excited state transition. In effect, the probability of an electron returning to a particular vibrational energy level in the ground state is similar to the probability of that electron's position in the ground state before excitation. This concept, known as the **Mirror Image Rule.**

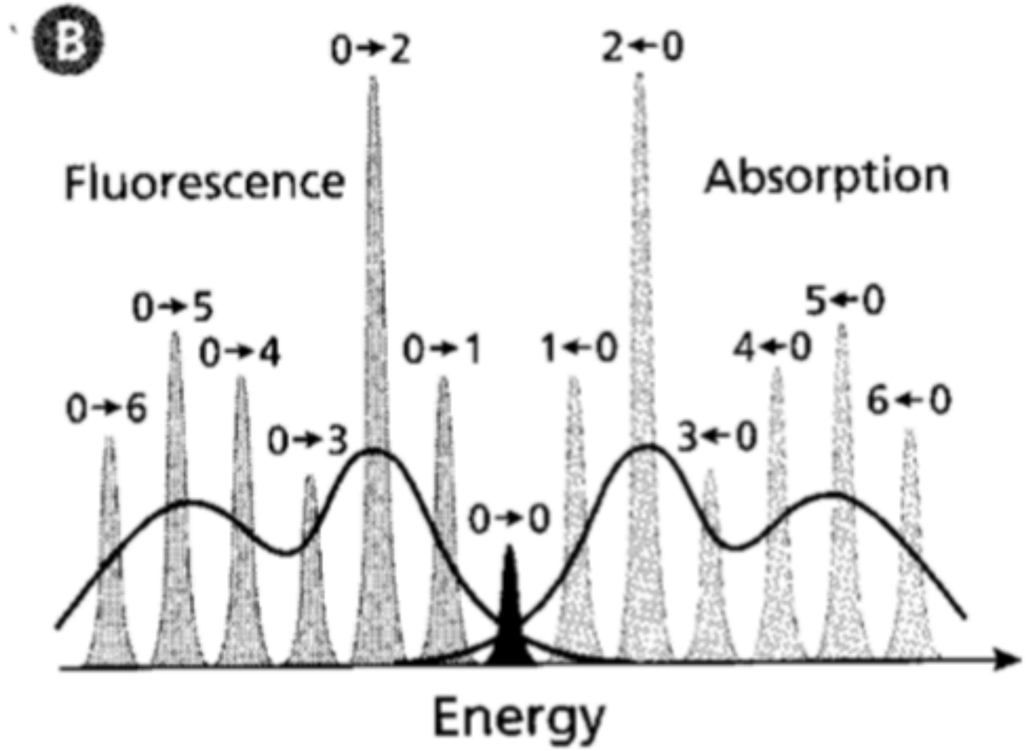
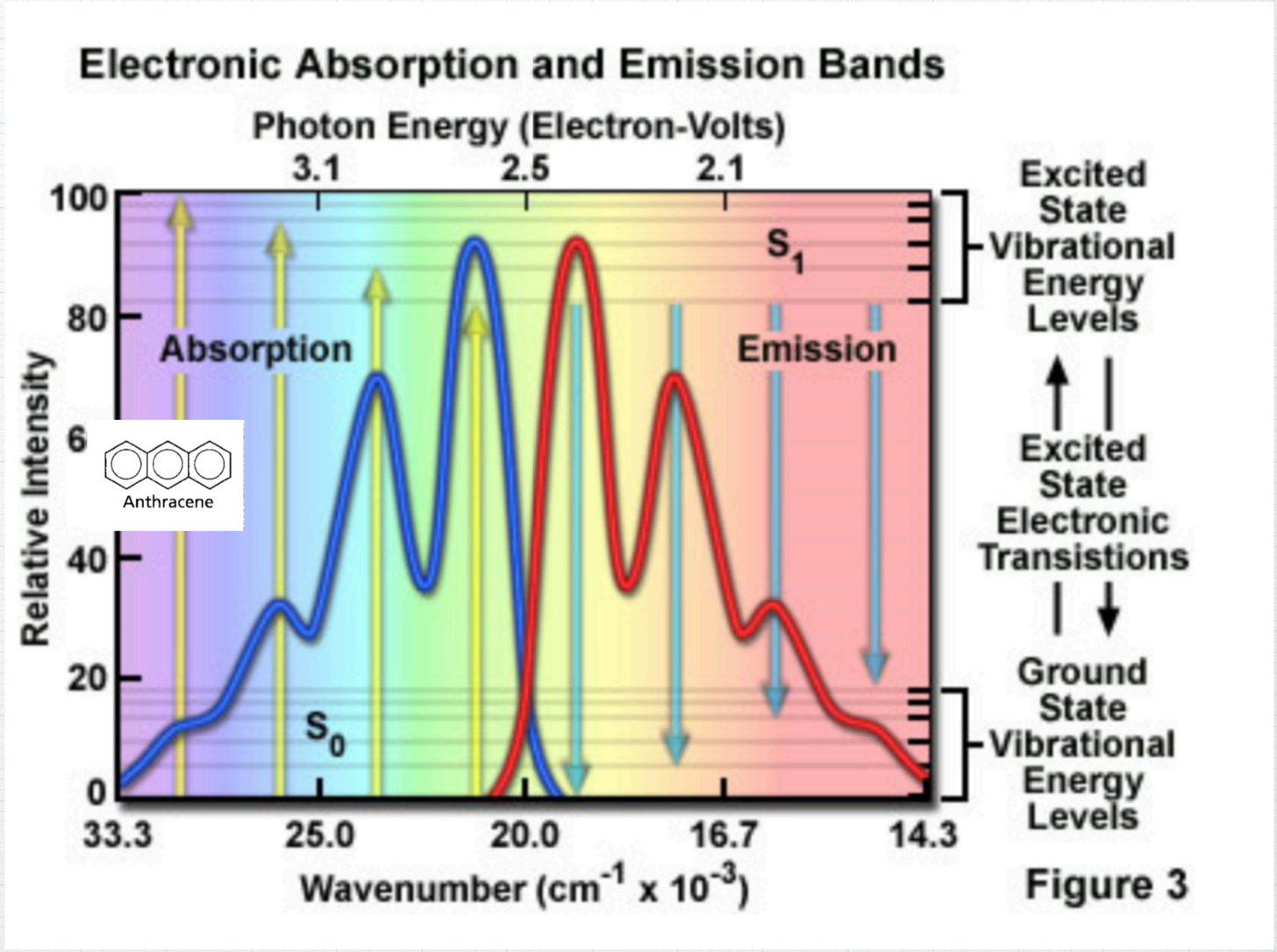


Figure 8.4 (A) Potential energy diagram showing two electronic states, ground state S_0 and excited state S_1, with vibrational levels (v) and (B) associated absorption and fluorescence spectra.



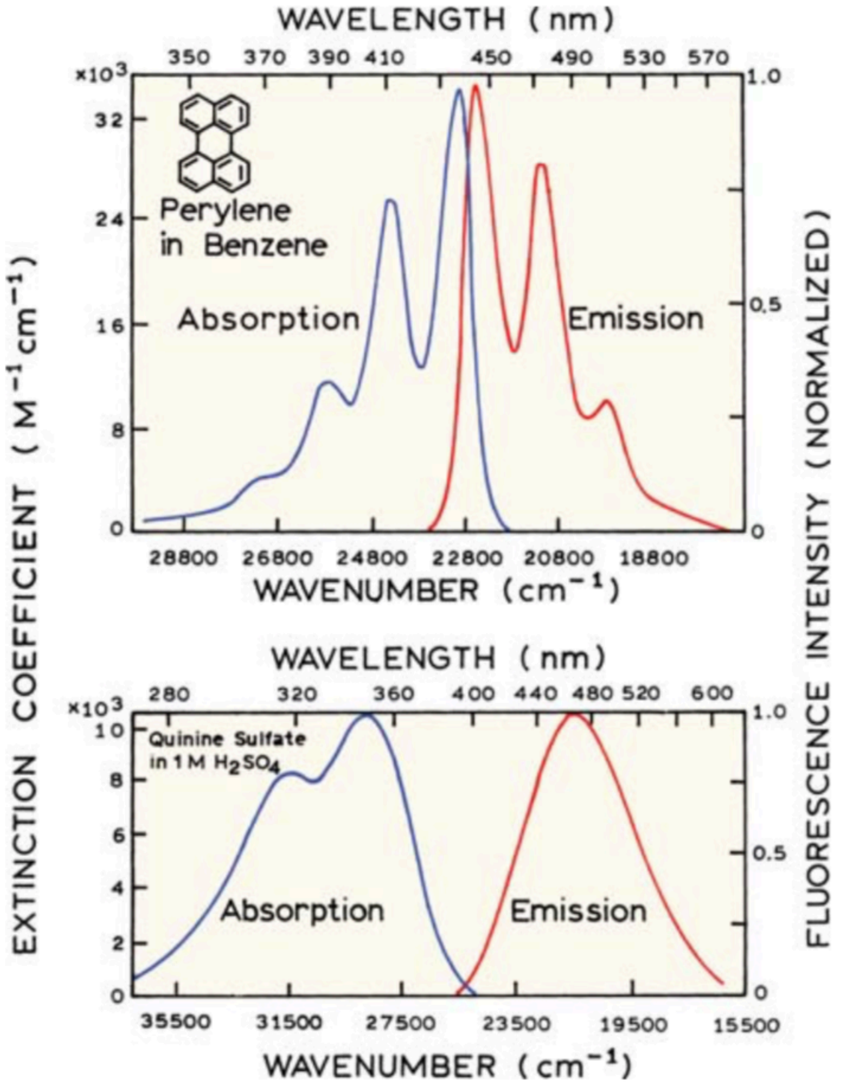
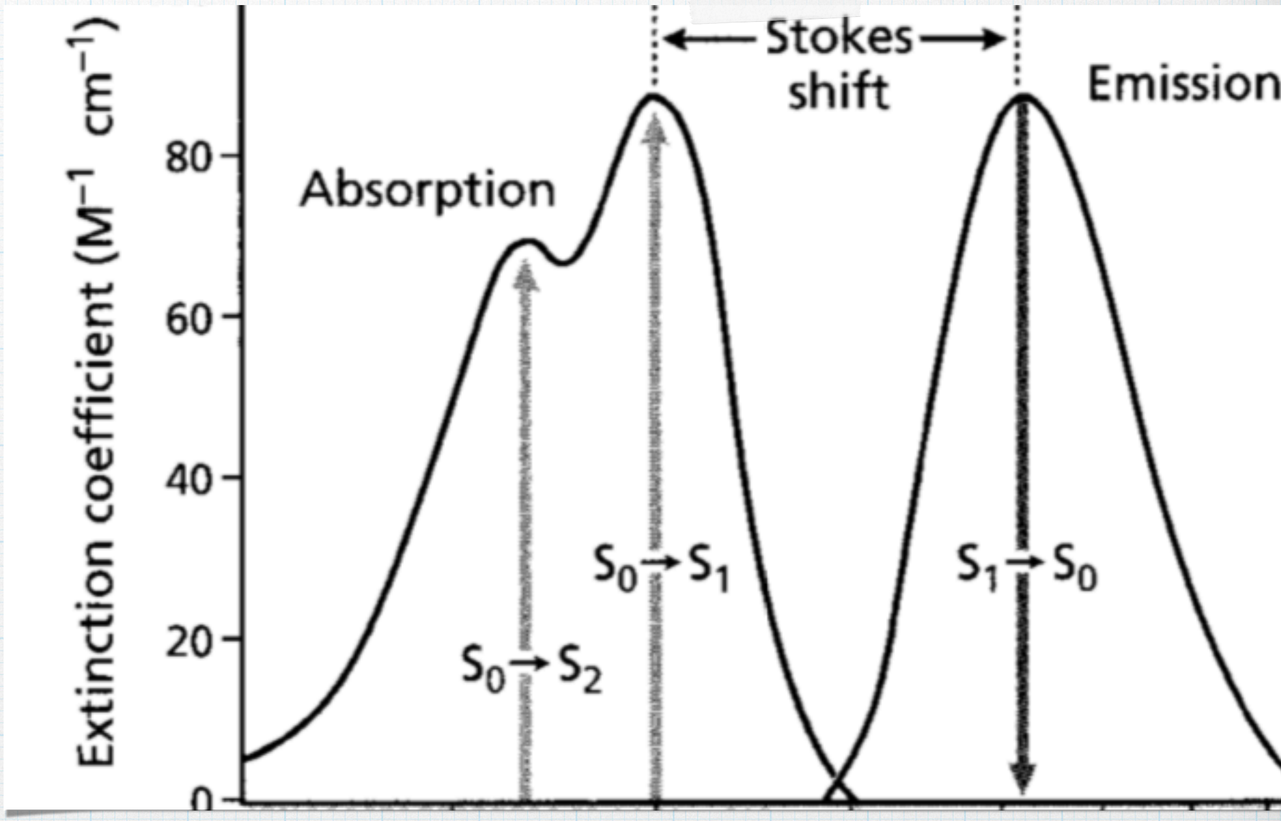


Figure 1.3. Absorption and fluorescence emission spectra of perylene and quinine. Emission spectra cannot be correctly presented on both the wavelength and wavenumber scales. The wavenumber presentation is correct in this instance. Wavelengths are shown for convenience. See Chapter 3. Revised from [5].

Exception of Mirror Image Rule

Quinine does not adhere to the mirror image rule as is evident by inspecting the single peak in the emission spectrum (at 460 nanometers), which does not mirror the two peaks at 310 and 350 nanometers featured in the bimodal absorption spectrum. The shorter wavelength ultraviolet absorption peak (310 nanometers) is due to an excitation transition to the second excited state (from **S(0)** to **S(2)**) that quickly relaxes to the lowest excited state (**S(1)**). As a consequence, fluorescence emission occurs exclusively from the lowest excited singlet state (**S(1)**), resulting in a spectrum that mirrors the ground to first excited state transition (350 nanometer peak) in quinine and not the entire absorption spectrum.



The effect of solvent on the fluorescence spectra

- The effect of solvent and environment may be due to several factor
  - Solvent polarity and viscosity
  - Rate of solvent relaxation
  - Probe conformational changes
  - Rigidity of the local environment
  - Internal charge transfer
  - Proton transfer and excited state reaction
  - Probe-Probe interaction.

Effect of solvent

Typically, the fluorophore has a larger dipole moment in the excited state than the ground state. Solvent shifts the emission to lower energy due to stabilization of the excited state by the polar solvent molecule. As the solvent polarity is increased , this effect becomes larger.

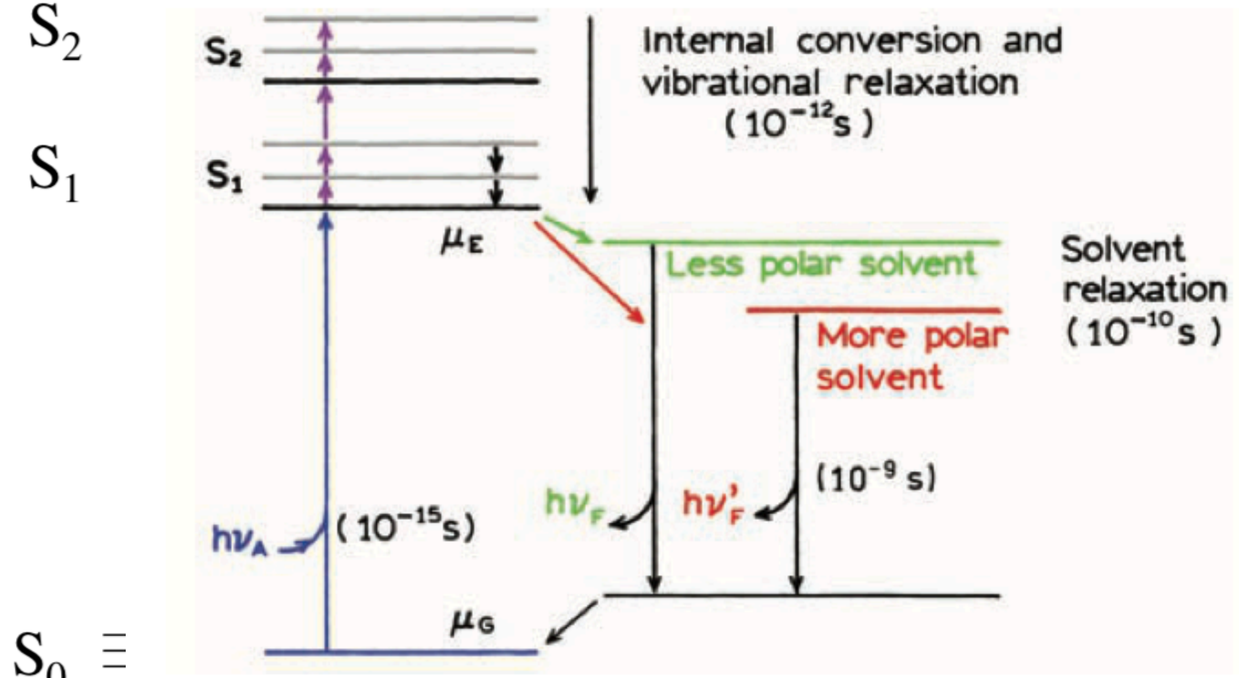
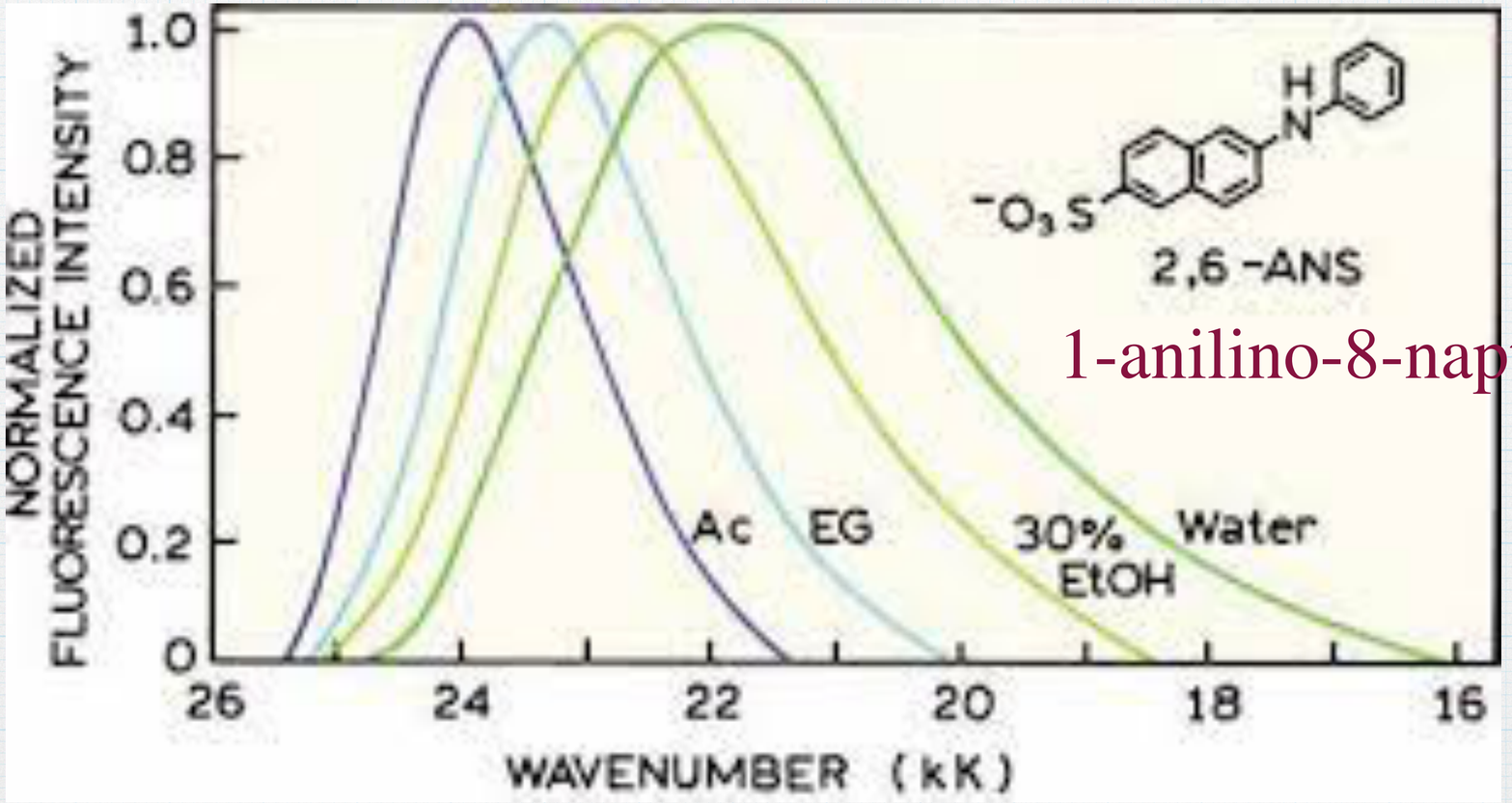


Figure 6.1. Jablonski diagram for fluorescence with solvent relaxation.



1-anilino-8-napthalene sulfonate

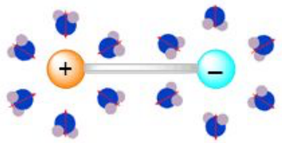
Interaction of the chromophore with the surrounding solvent molecules may occur prior to emission.

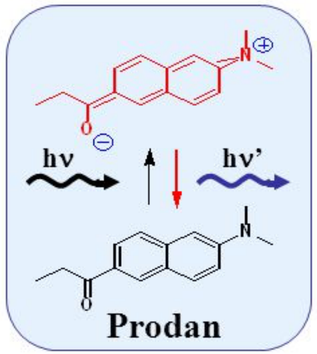
Environment-sensitive probes

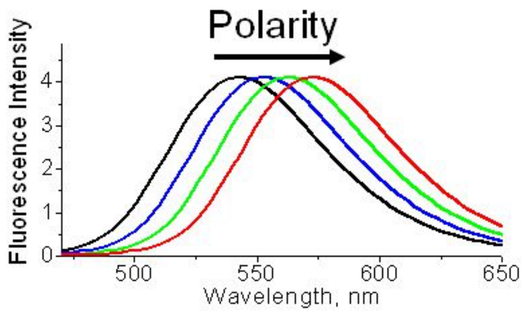
Oil:
nonpolar

Poor solvation

Water:
Polar

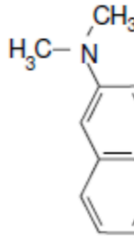






➢**Environment-sensitive probes change their color with the change of polarity**

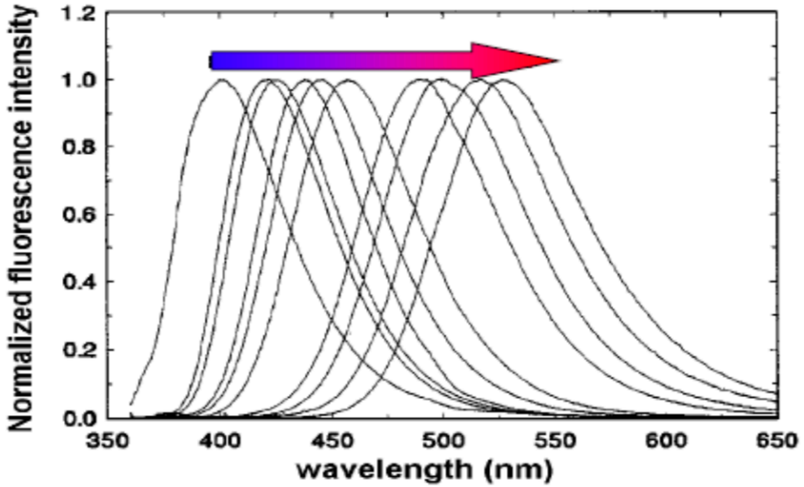
Fluorescence spectra of Prodan



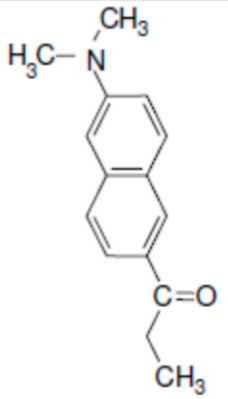
Emission spectra of prodan in different solvents:

heptane

water



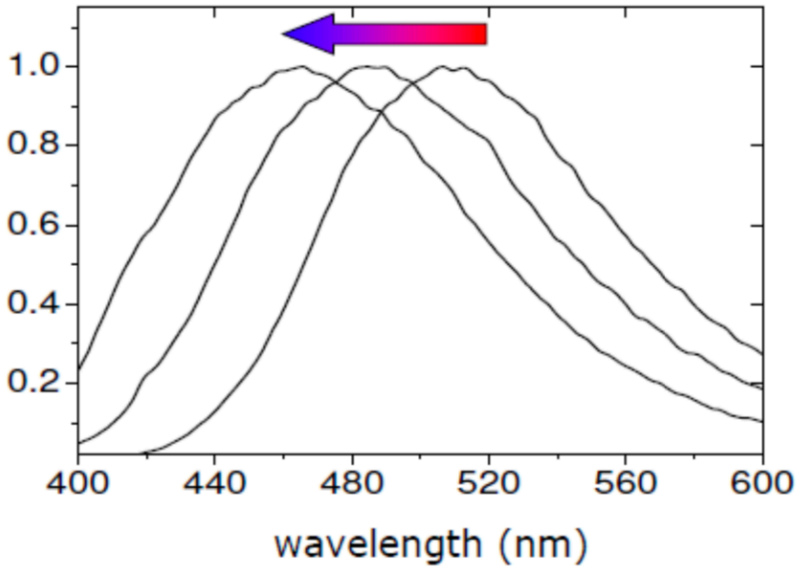
Fluorescence spectra of Prodan



Emission spectra of prodan at different temperatures:

100 K

300 K



Decrease of temperature → increase of viscosity → increasing fluorescence contributions of non-relaxed states → blue-shift

Factors affecting fluorescence

Conjugation: Molecule must have unsaturation i.e. it must have π electrons so that UV/vis radiation can be absorbed. If there is no absorption of radiation, there will not be fluorescence.

Nature of substituent groups :Electron donating groups like amino, hydroxyl groups enhance fluorescence activity. Electron withdrawing groups like Nitro, carboxylic group reduce fluorescence. Groups like SO_3H or on NH_4^+ have no effect on fluorescence intensity.

Effect of temperature : Increase in temperature leads to increase in collisions of molecules and decrease in fluorescence intensity while decrease in temperature leads to decrease in collisions of molecules and increased fluorescence intensity.

Factors affecting fluorescence

Viscosity : Increase in viscosity leads to decreased collisions of molecules which will enhan fluorescence intensity while decrease in viscosity causes increased collisions of molecul which cause decreased fluorescence

intensity.

Oxygen :Oxygen decreases the Fluorescence intensity in two ways: Oxidises fluorescenc substances to non fluorescence substances. It quenches fluorescence because of paramagneti properties.

Effect of pH: Aniline: Neutral or alkaline medium shows visible fluorescence while acidic conditions give fluorescence in UV region only.

Phenols : Acidic conditions do not give fluorescence while alkaline conditions gives good fluorescence.