

Today's class:

Bioluminescence part 1

This lecture follows the materials from the following books

- *Physical Biochemistry by David Sheehan, 2nd Ed, Wiley, 2009*
- *Physical Chemistry for Life Sciences, by PW Atkins and JD Paula, Oxford, 2006*

Luminescence is spontaneous emission of light

Luminescence

Emission of light from electronically excited species not in thermal equilibrium with its environment

Also called 'Radiative decay'

Photoluminescence

Emission of light due to excitations by photon



Chemiluminescence

Emission of light due to chemical reactions



Here we will focus on different types of photoluminescence of biomolecules

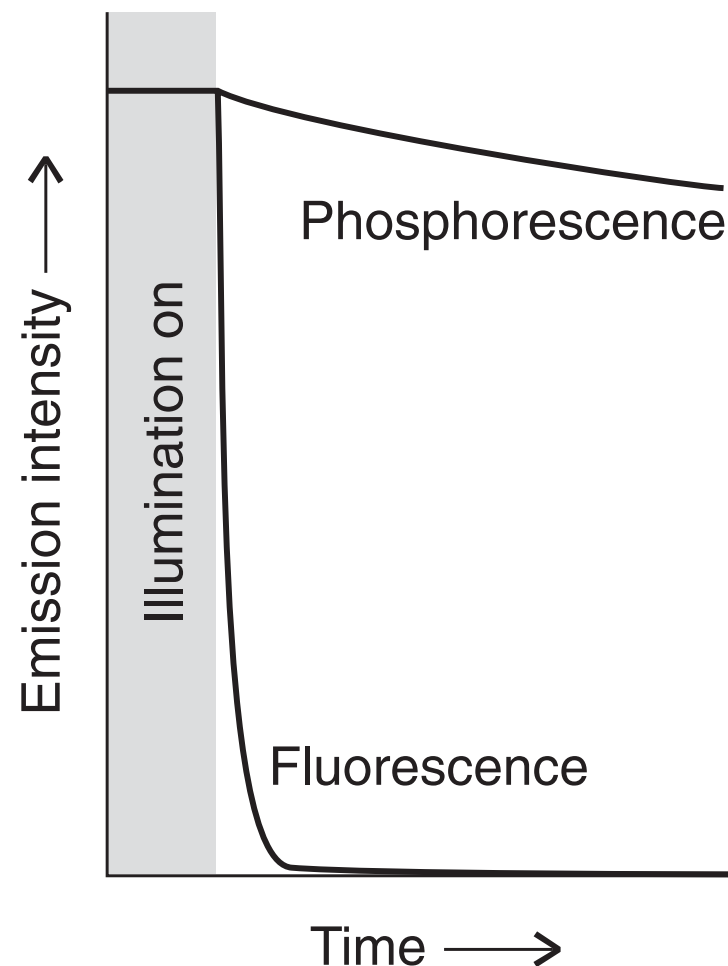
Photoluminescence is divided into two categories

Fluorescence

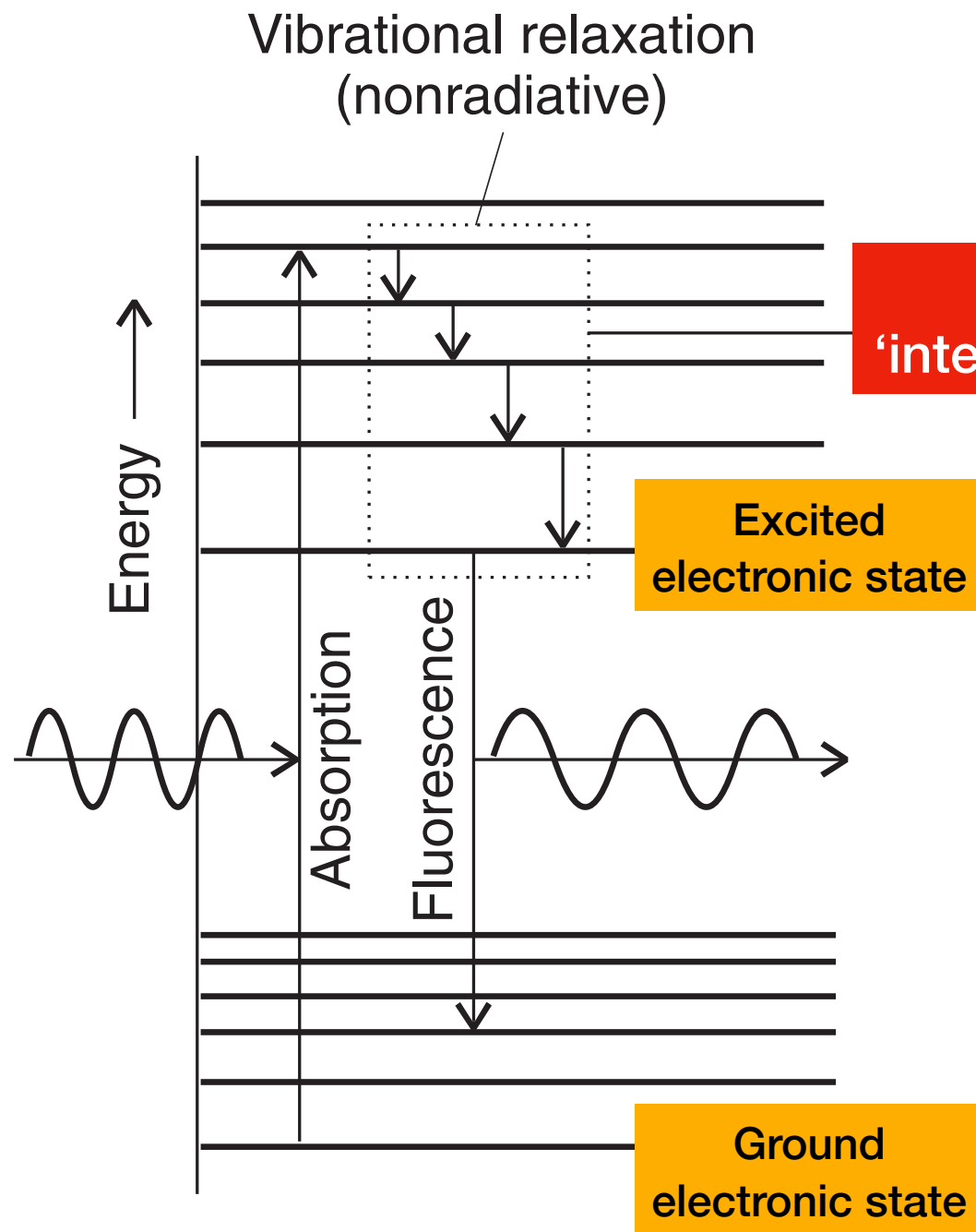
the process in which a molecule absorbs a photon of light and then almost instantaneously re-emits a photon of light of lower energy.

Phosphorescence

the process in which a molecule emits a photon from an electronically excited state but the emission persists for long periods



Physical basis of fluorescence



- Lifetime of the excited state ~ pico to nanoseconds
- Separation of the ground states of the electronic states (the lowest horizontal line in each set) is 10 to 100 times greater than the separation of the vibrational levels.
- The emitted photon is always of longer wavelength than the incident photon

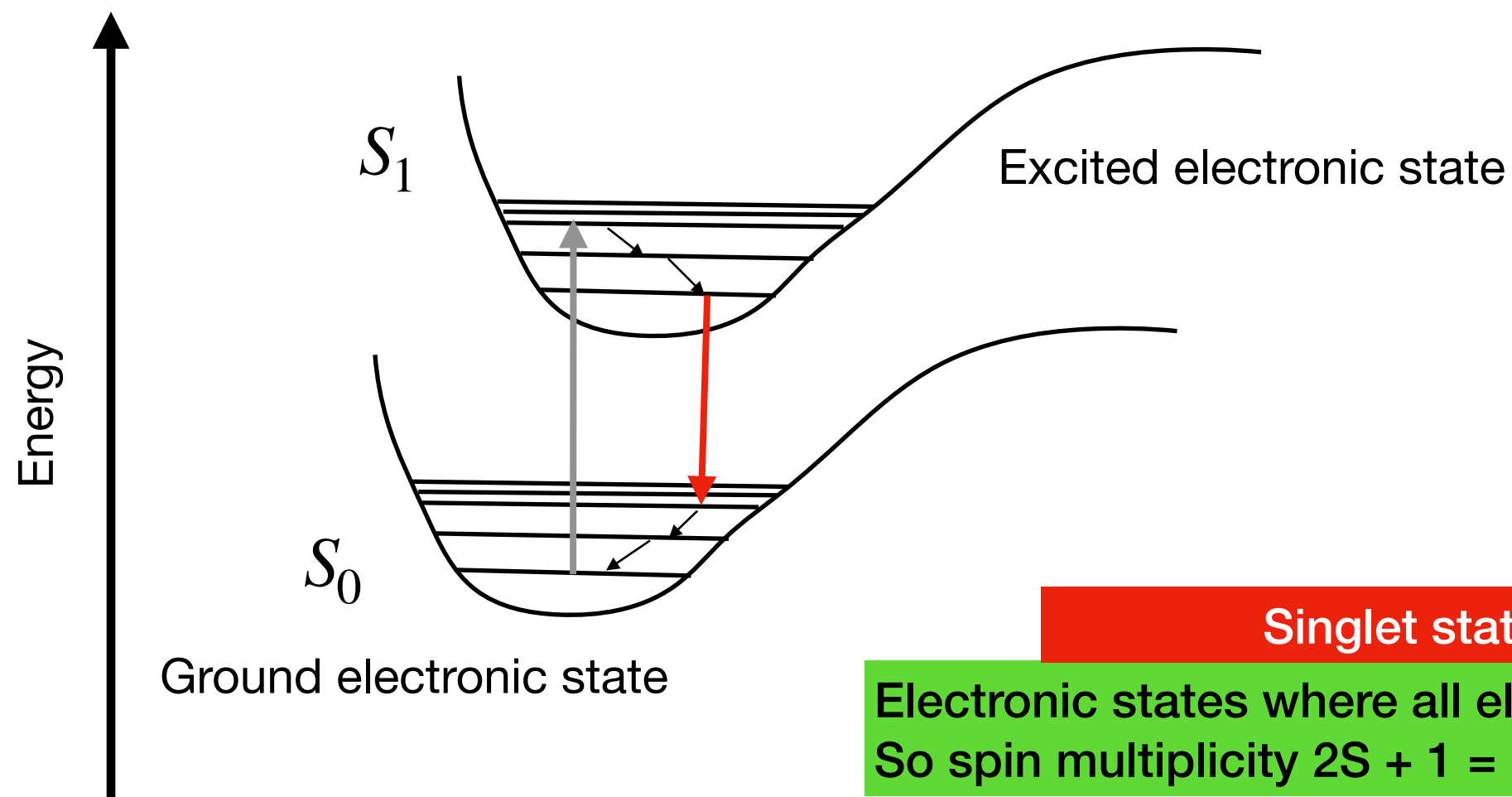
$$h\nu_{excitation} > h\nu_{emission}$$

$$\Rightarrow \lambda_{fl} > \lambda_{exc}$$

Such diagrams are called
Jablonski diagrams

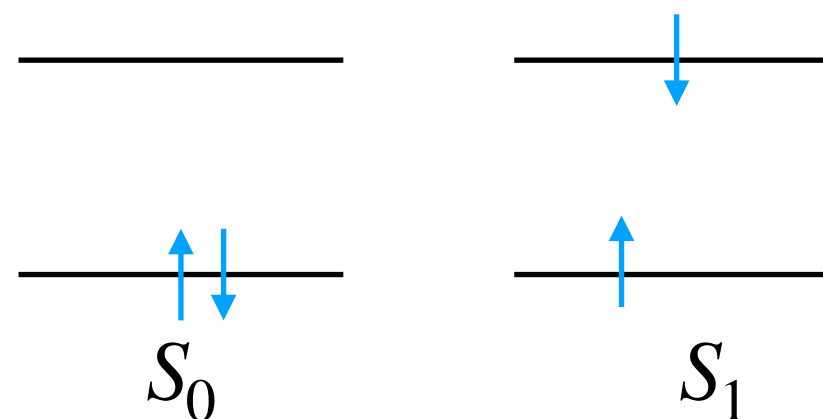
Features of fluorescence emission

Fluorescence only happens between singlet electronic states



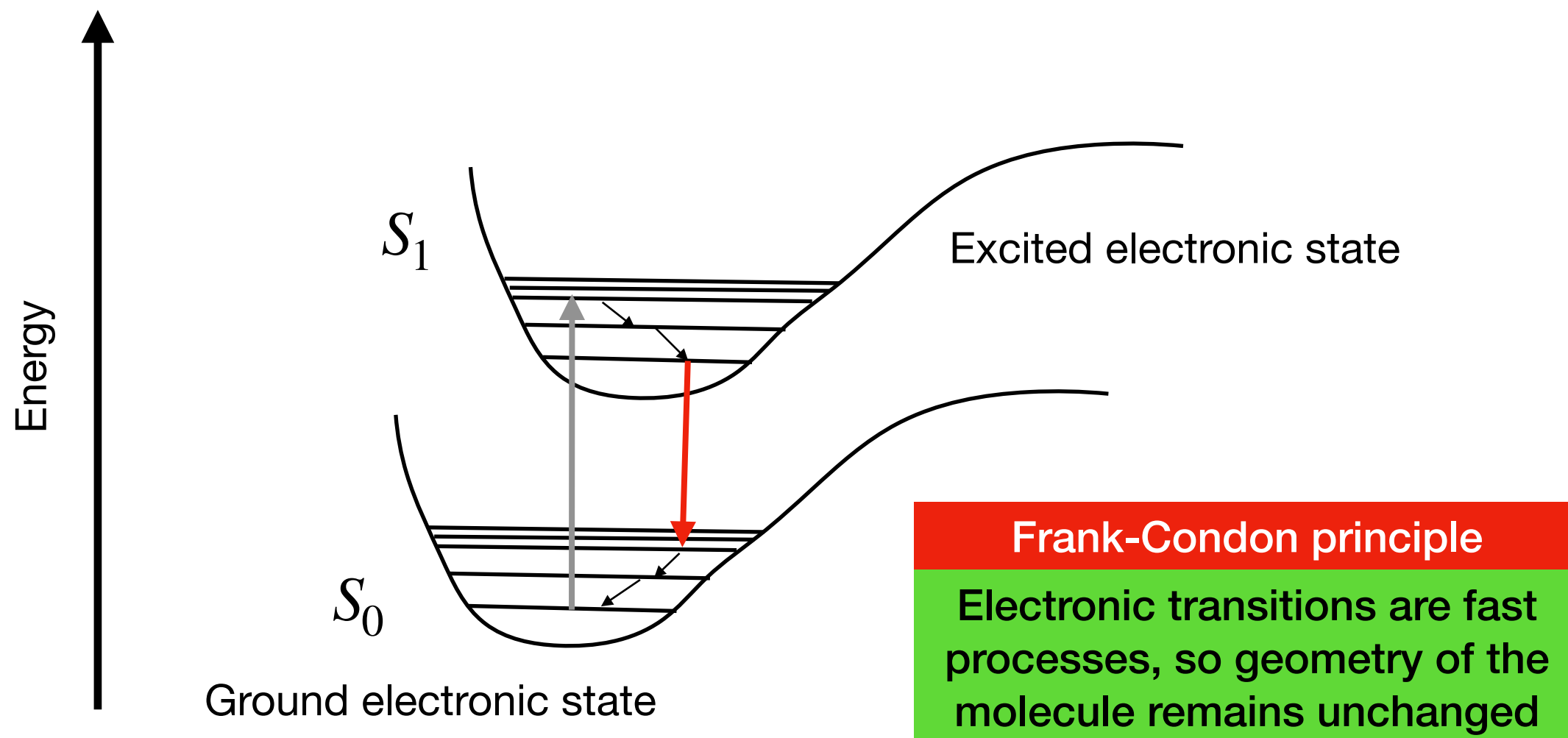
Singlet states

Electronic states where all electrons are paired.
So spin multiplicity $2S + 1 = 1$. ($S = 1/2 + -1/2 = 0$)



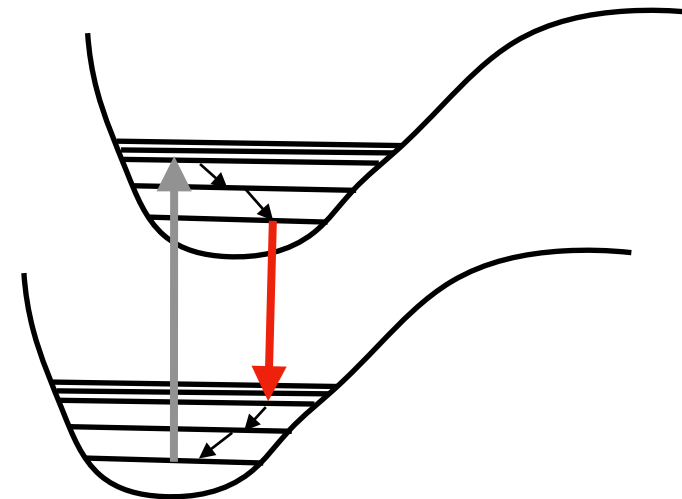
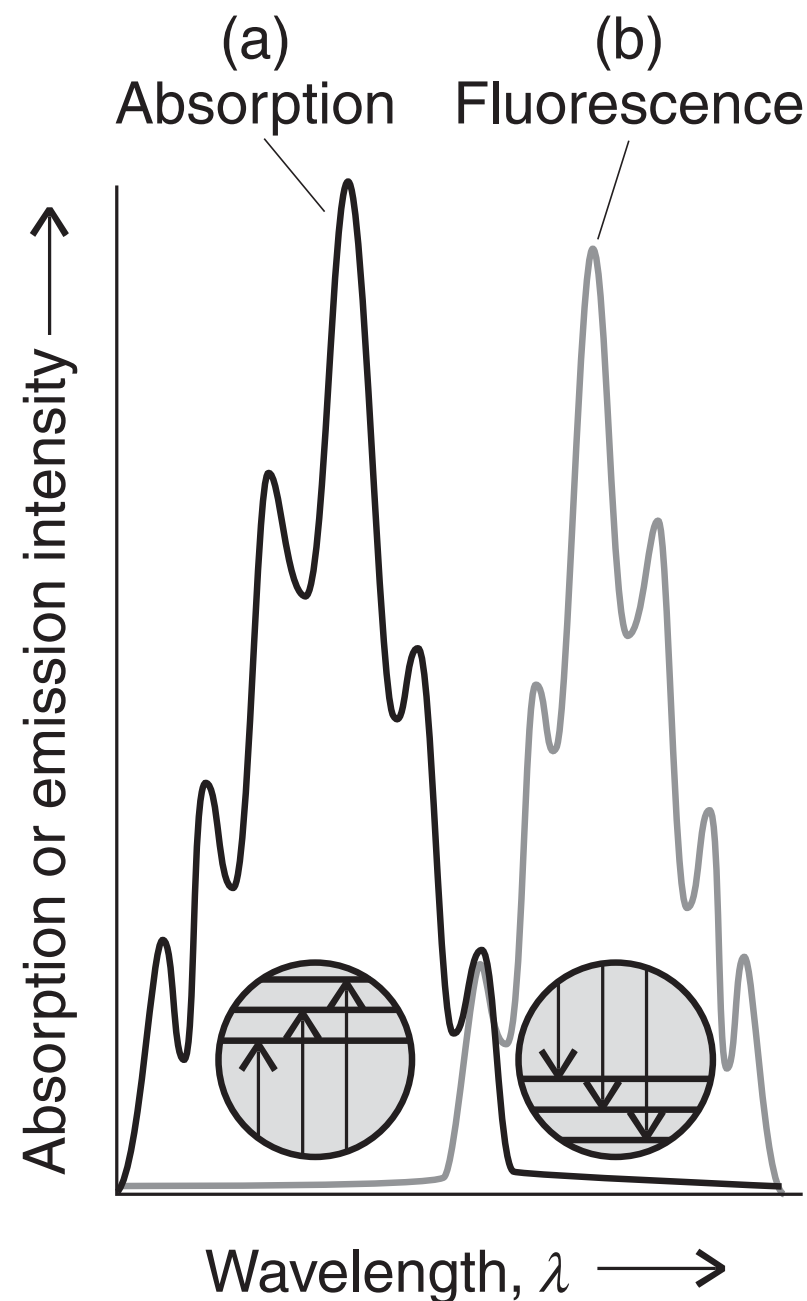
Features of fluorescence emission

Like absorption, the fluorescence emissions are 'vertical' transitions



Features of fluorescence emission

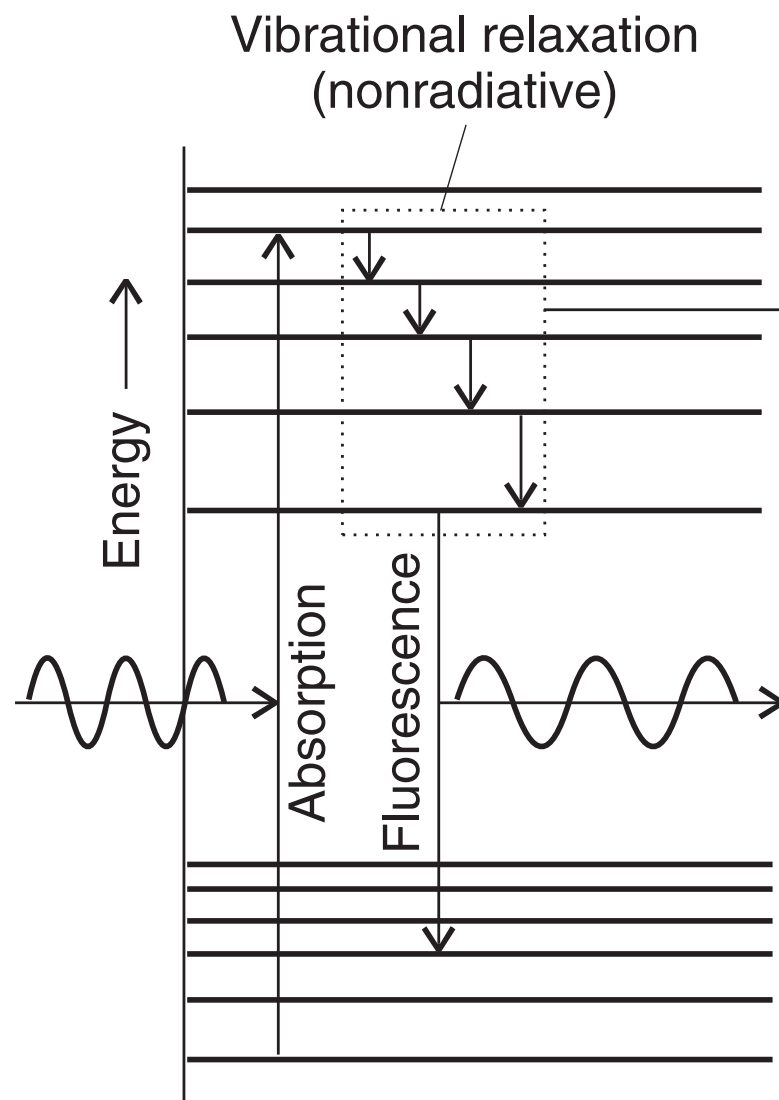
Fluorescence emission spectrum resemble a mirror image of the corresponding absorption spectrum



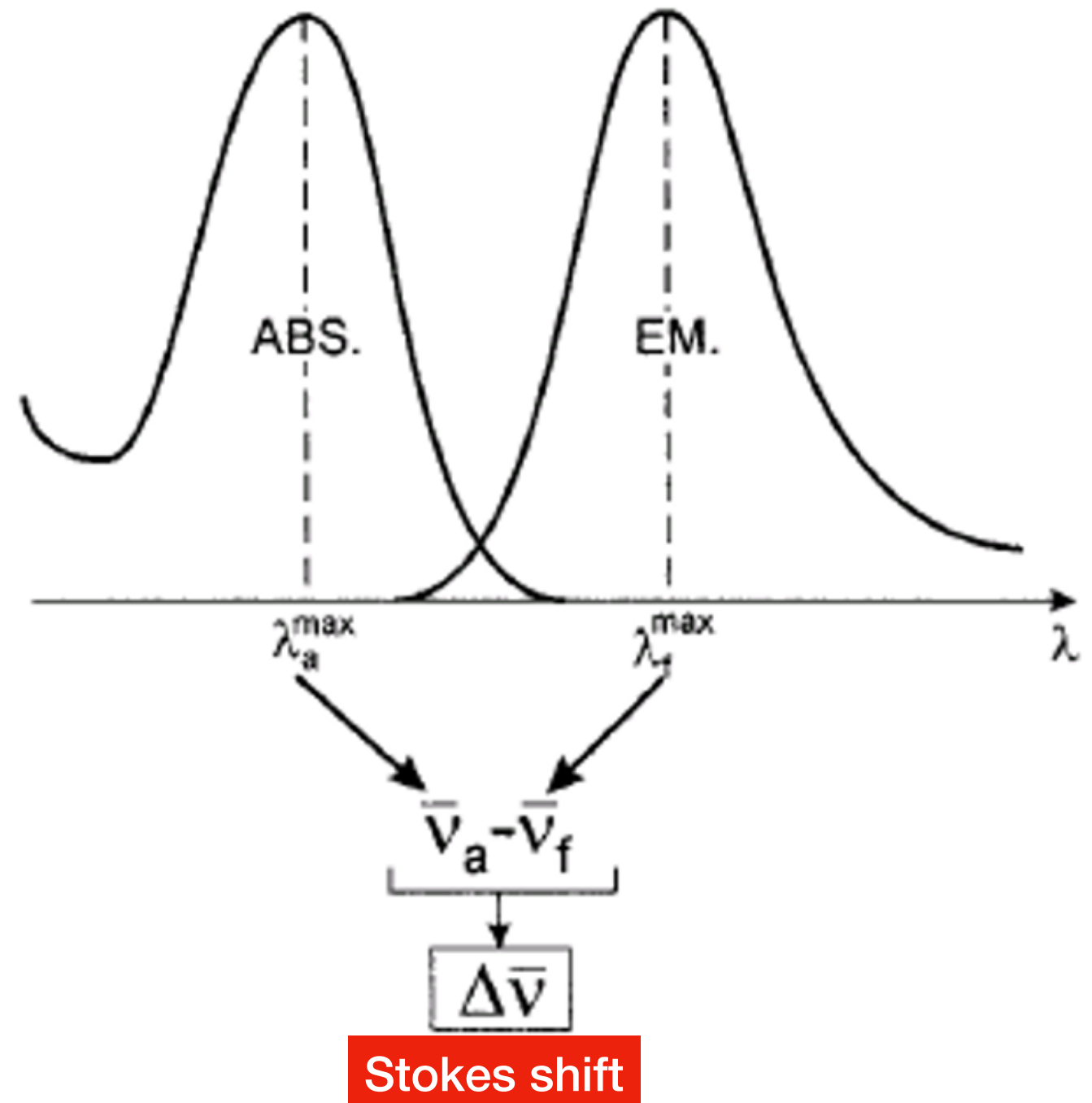
- Absorption spectrum = excitations to different vibrational energy levels of the excited electronic state.
- So, it captures the vibrational structure of the excited electronic state.
- Fluorescence spectrum = de-excitations to different vibrational energy levels of the ground electronic state.
- So, it captures the vibrational structure of the ground electronic state.
- Because the vibrational structures of states of same symmetry are similar the two spectrum appear as mirror images

Features of fluorescence emission

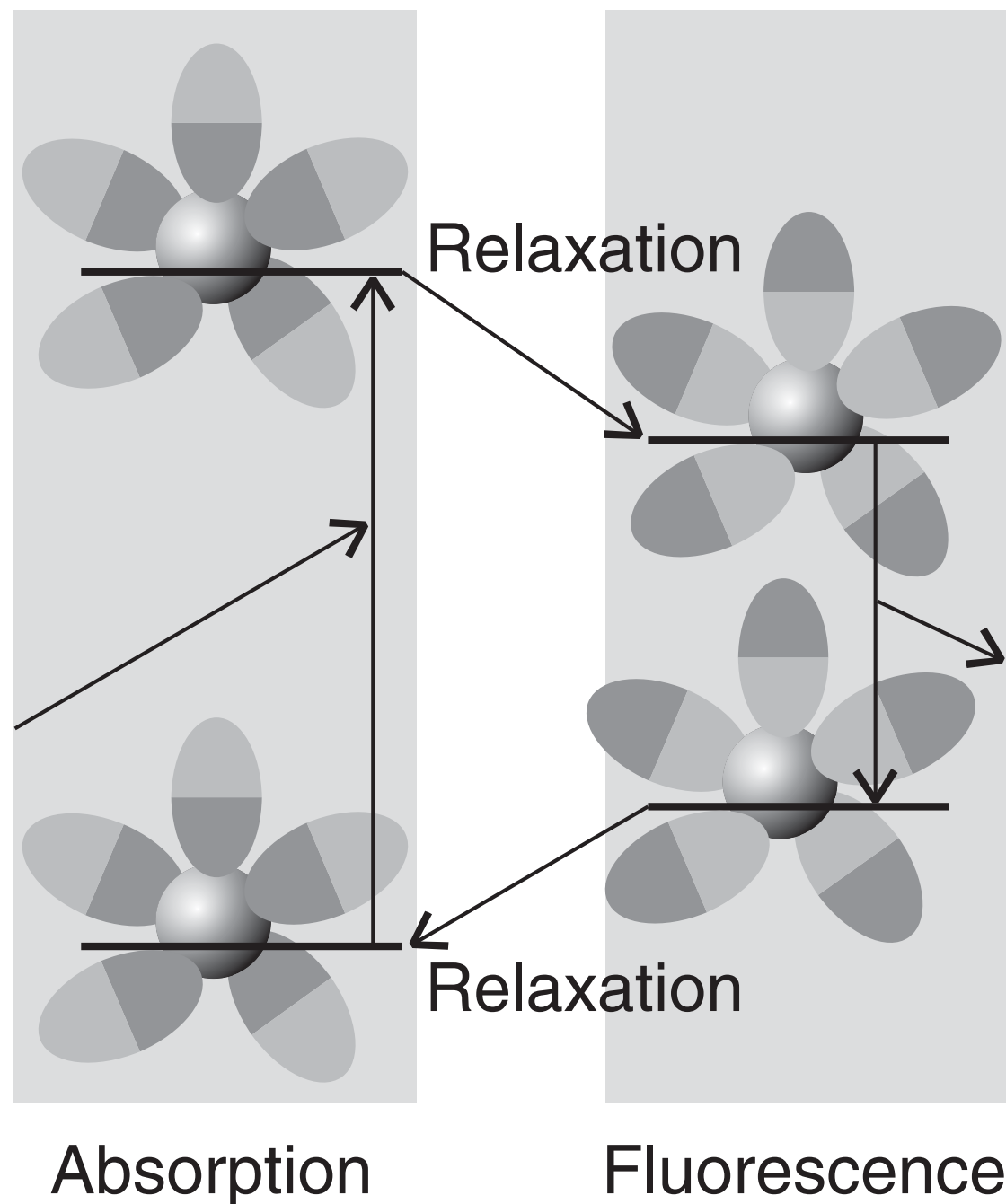
The Stokes shift is the gap between the maximum of the first absorption band and the maximum of the fluorescence spectrum



The vibrational non-radiative decay through collisions with solvent and other molecules makes the emitted photon lower energy than the absorbed photon

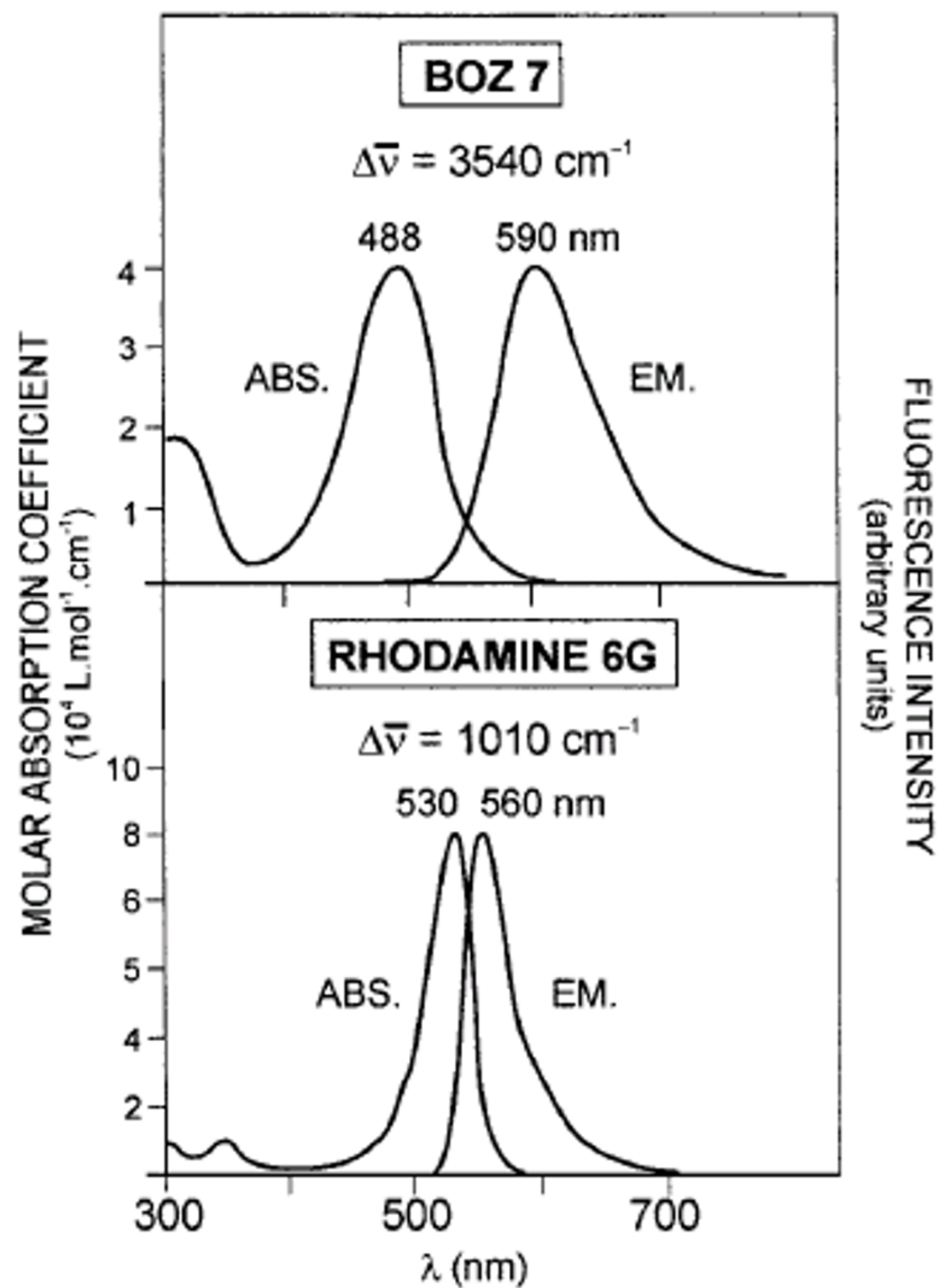


How can the solvents influence fluorescence?



- Absorption occurs so fast that the solvent arrangement characteristic of the ground electronic state of the molecule cannot change.
- However, before fluorescence occurs, the solvent molecules relax into a new arrangement, and that arrangement is preserved during the subsequent radiative transition.
- This way new transition happens from a new solvent arrangement which contributes in the stokes shift.

Examples of fluorescence spectra

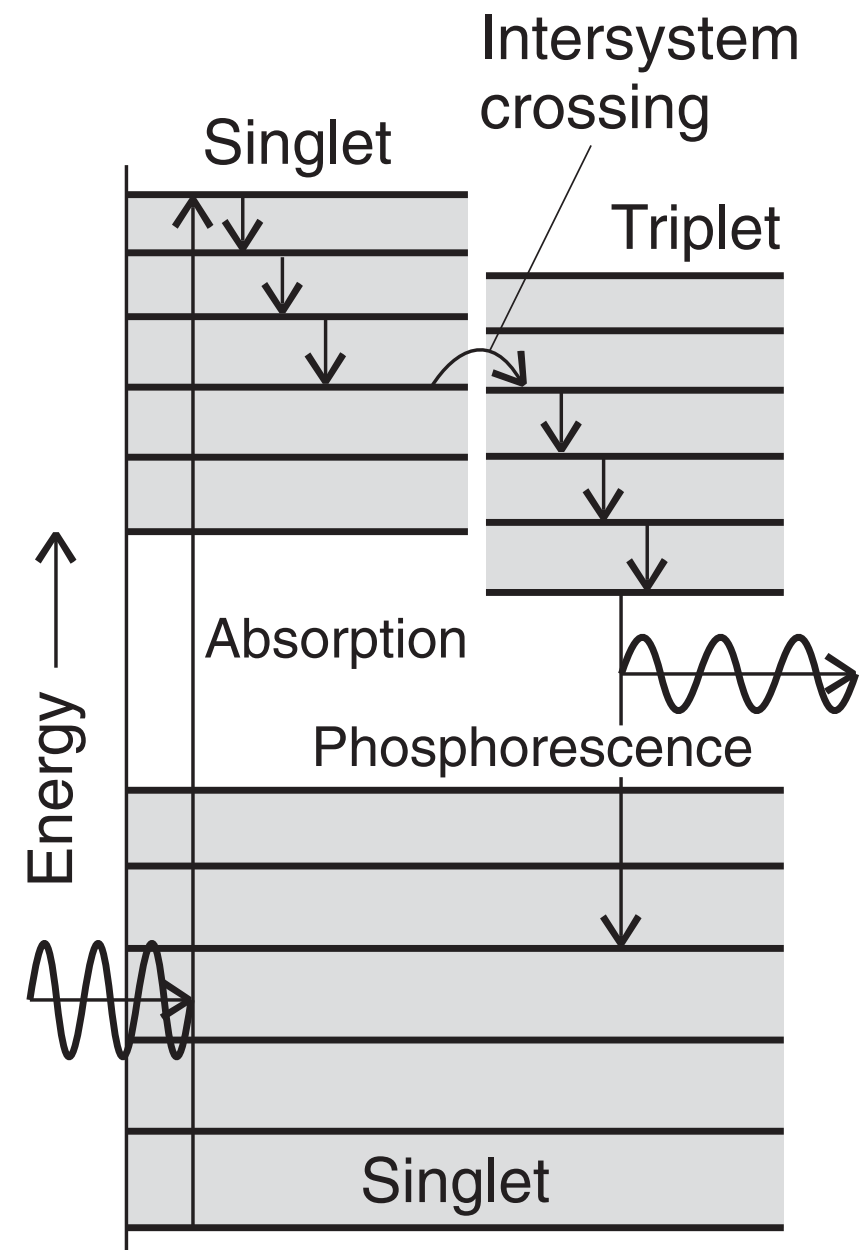
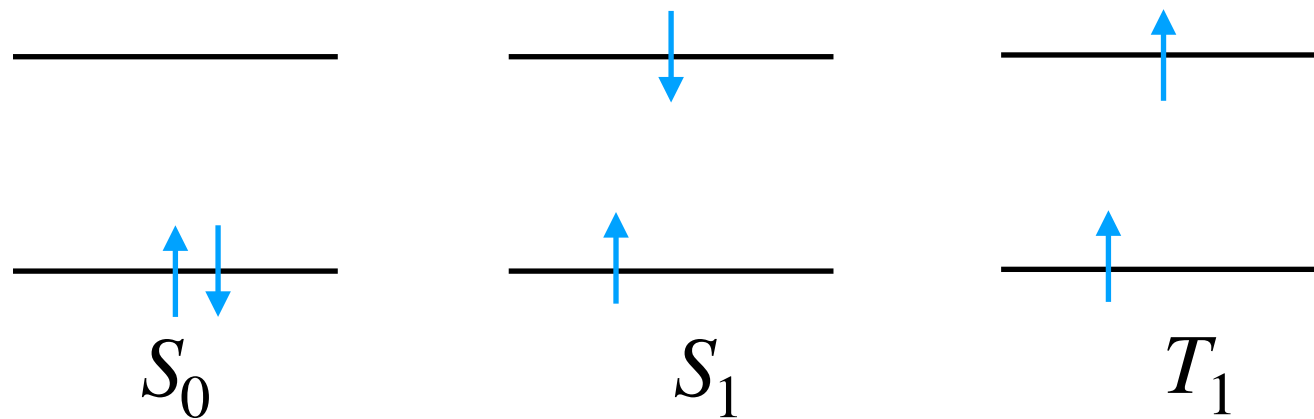


Physical basis of phosphorescence

Phosphorescence happens when Singlet and Triplet excited electronic states overlap via 'inter system crossing' (ISC)

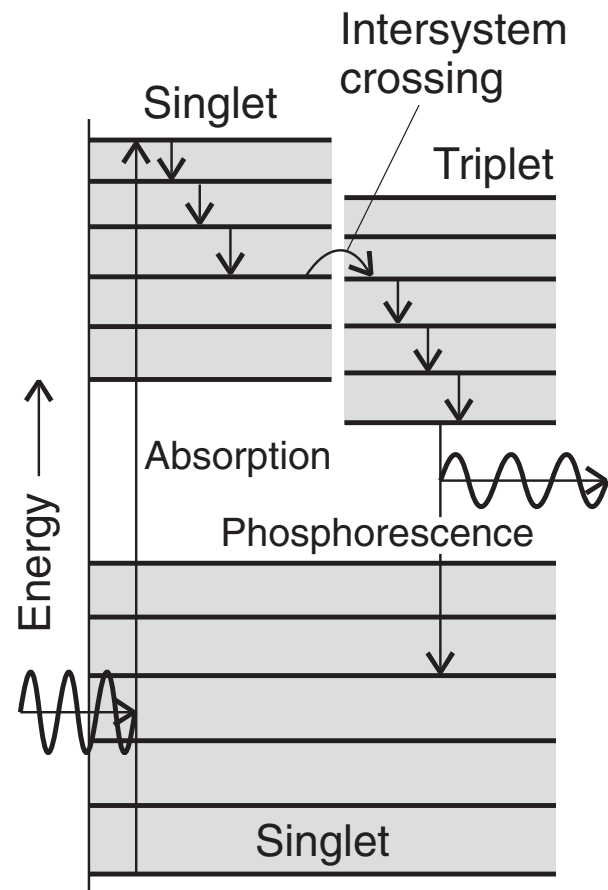
Triplet states

Electronic states where all electrons are unpaired.
So spin multiplicity $2S + 1 = 3$. ($S = 1/2 + 1/2 = 1$)



Physical basis of phosphorescence

The main difference between fluorescence and phosphorescence is the time taken by the process. Fluorescence is almost instantaneous but phosphorescence take much longer



- Normally $S \rightarrow T$ or $T \rightarrow S$ transitions are not allowed. A phenomenon called 'spin-orbit' coupling makes this happen.
- Phosphorescence is a very slow process for the low probability of this coupling to occur
- phosphorescence efficiency depend on the extent of spin-orbit coupling in the molecule
- Lifetime of the triplet excited state ~ micro to milli seconds or even fraction of seconds
- Rate of phosphorescence increases when heavy atoms, like S, Br, I etc. are present. They can facilitate spin-orbit coupling.