Today’s class:

Bioluminescence part 2

This lecture follows the materials from the following books

- *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

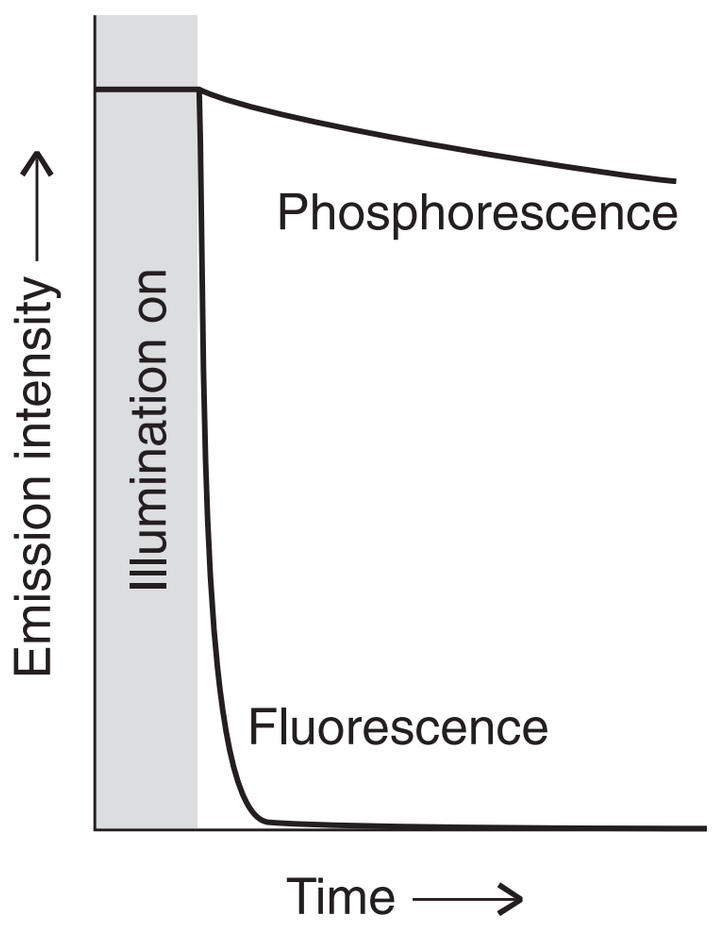
Photoluminescence is divided into two categories

Fluorescence

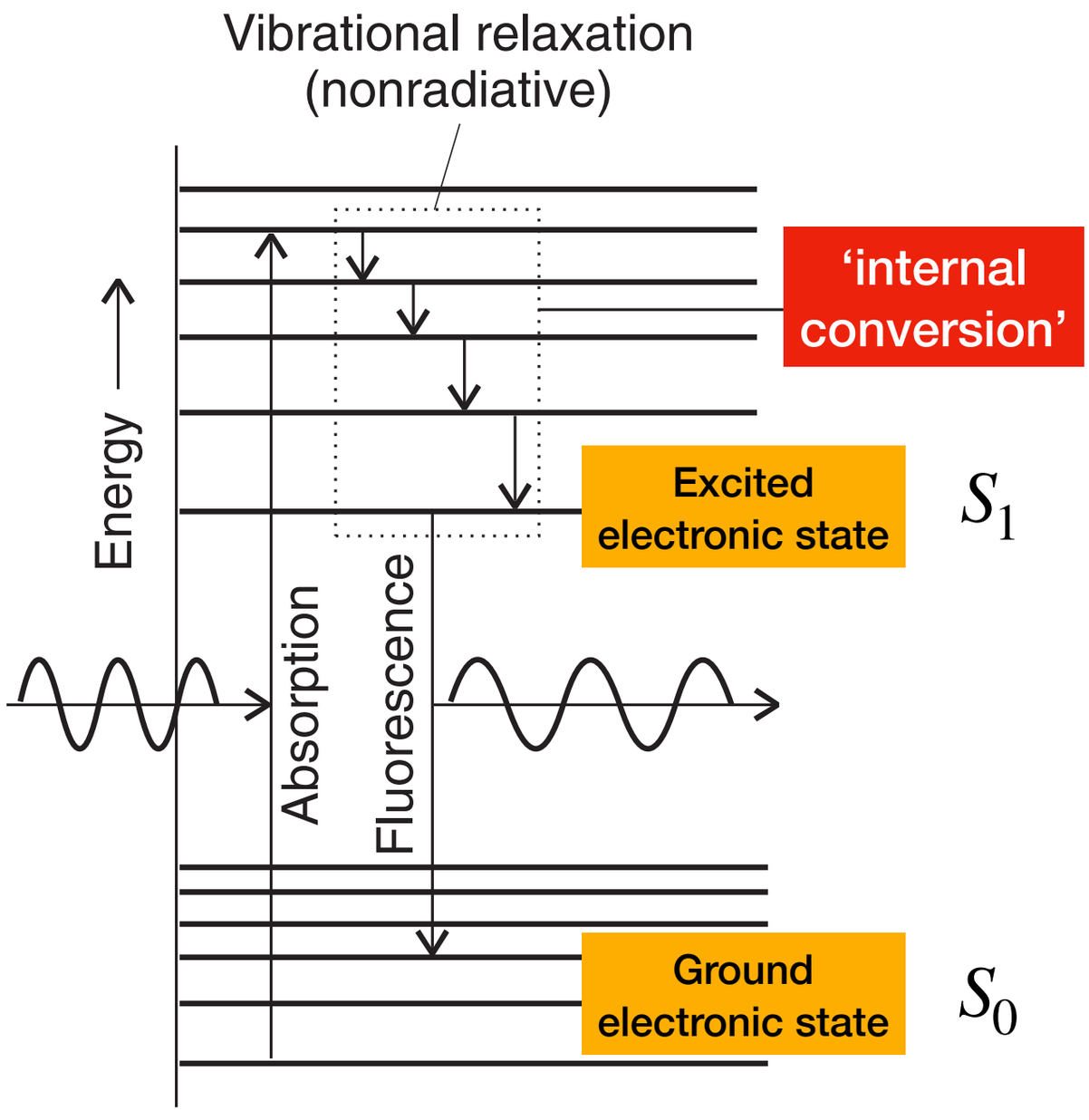
the process in which a molecule absorbs a photon of light and then almost instantaneously re-emits a photon of light of lower energy.

Phosphorescence

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

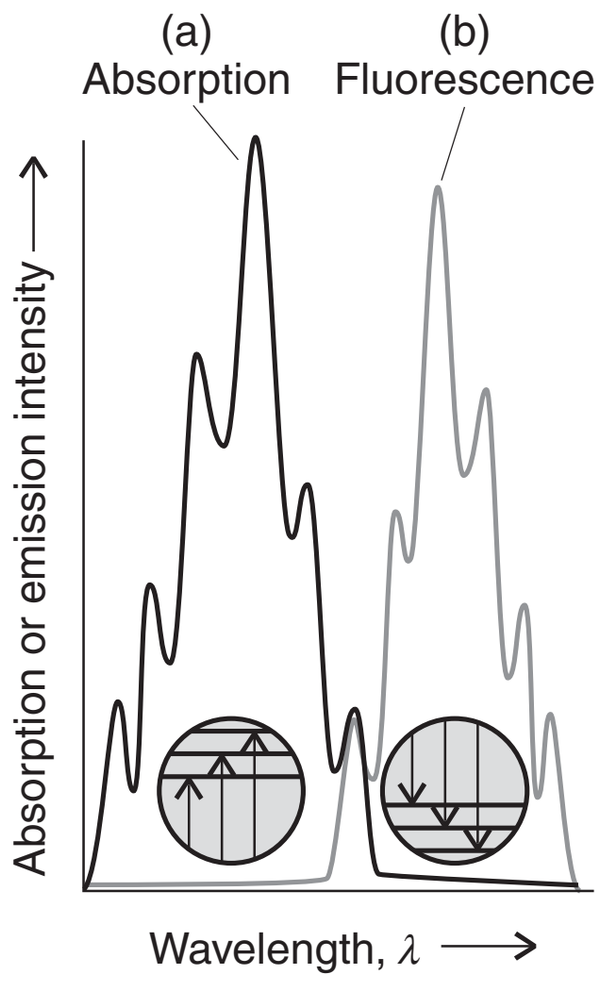


Physical basis of fluorescence



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

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



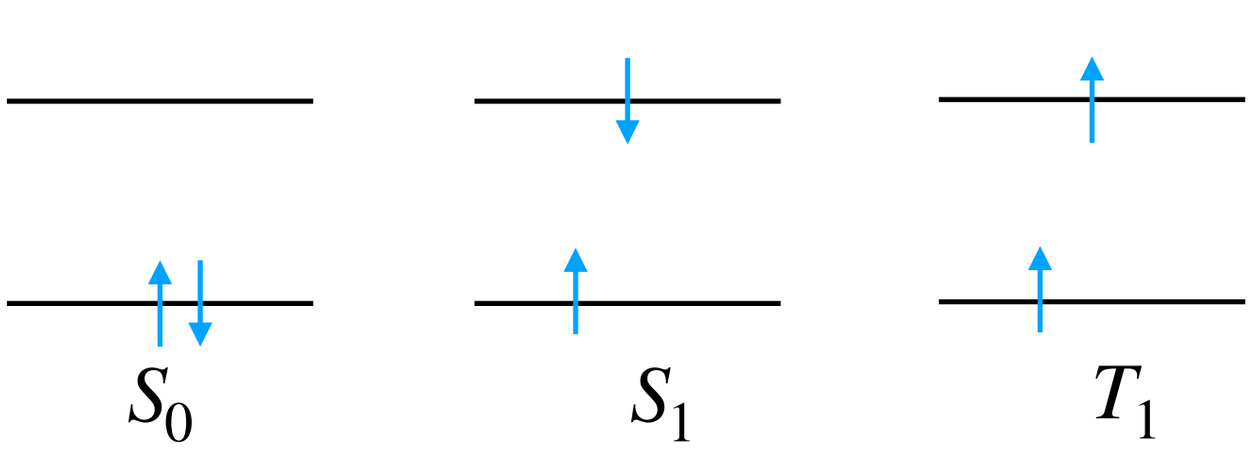
Mirror image relationship

Physical basis of phosphorescence

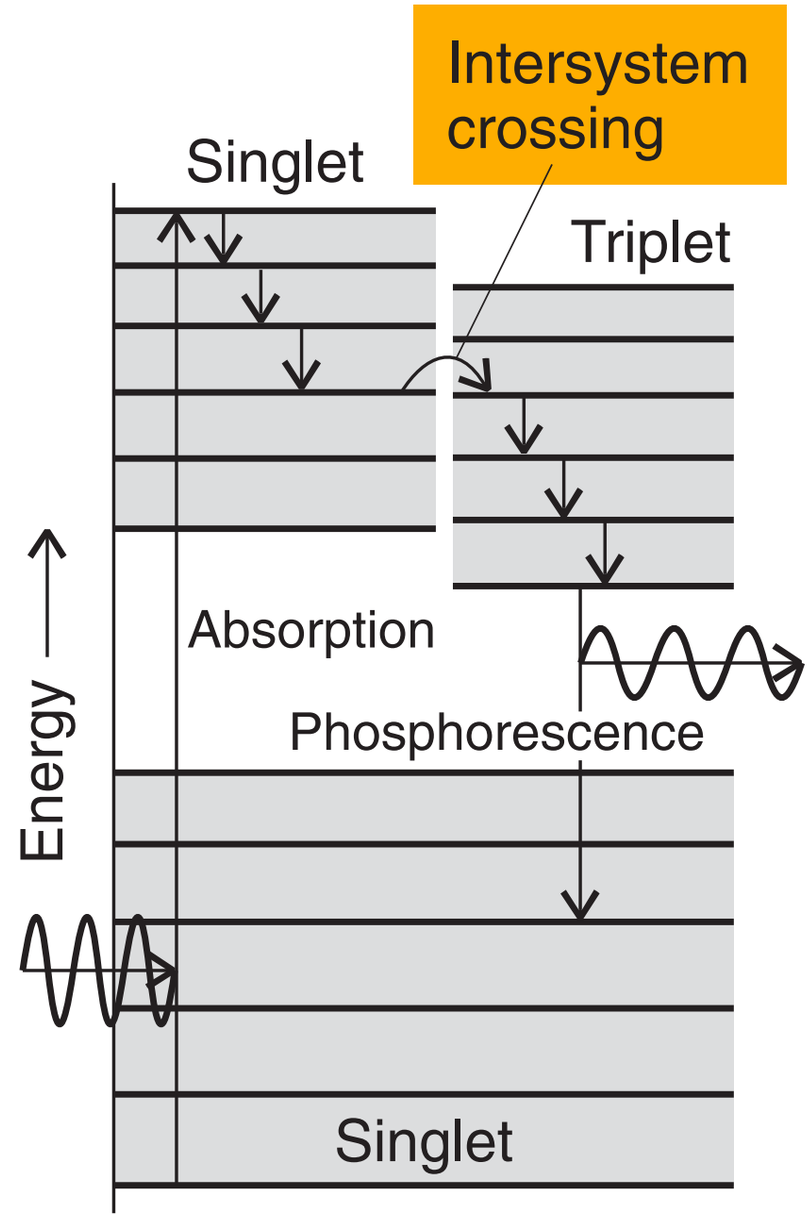
Phosphorescence happens when Singlet and Triplet excited electronic states overlap via ‘inter system crossing’ (ISC)

Triplet states

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



The main difference between fluorescence and phosphorescence is that fluorescence is almost instantaneous but phosphorescence take much longer



Photobiology

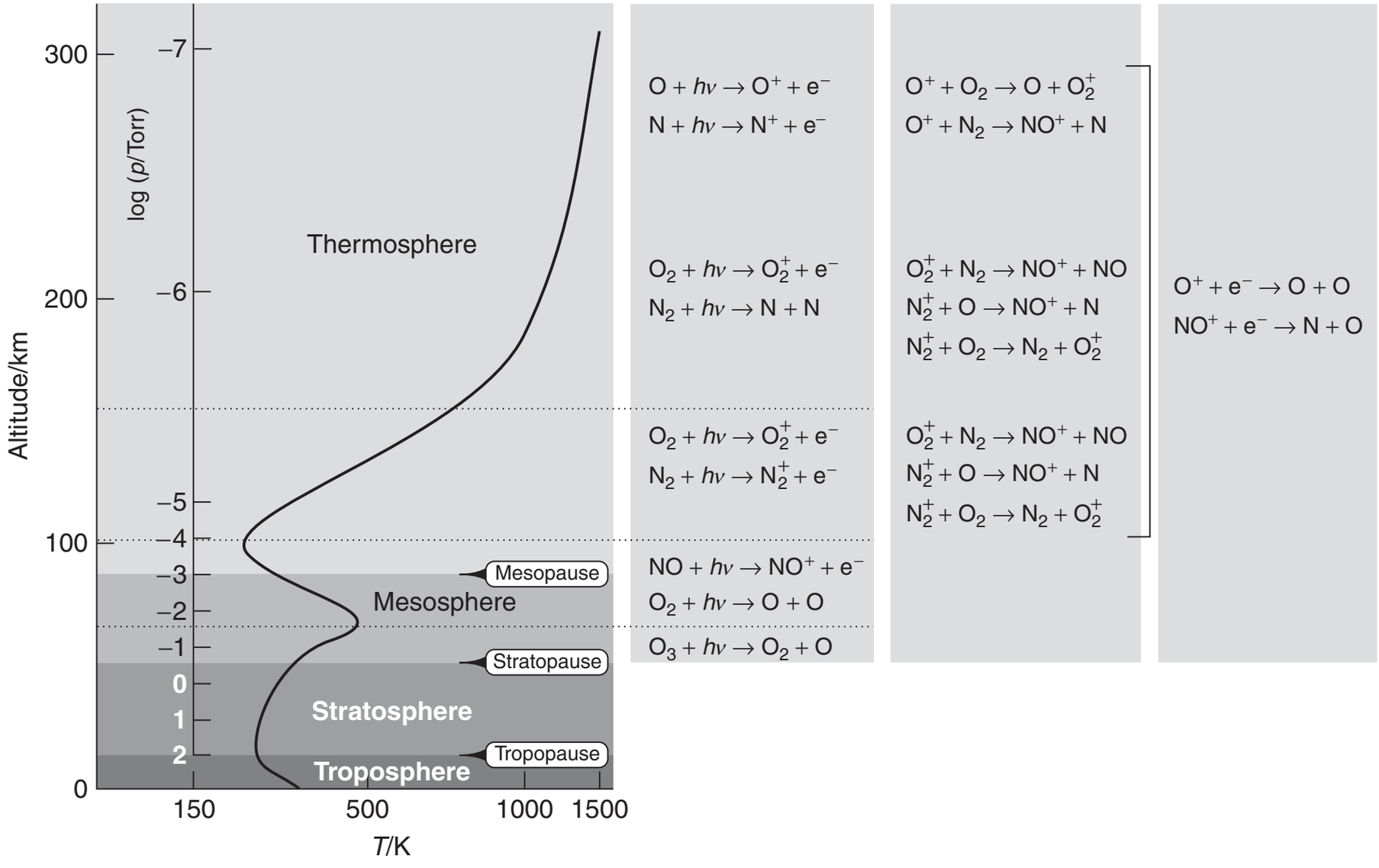
Interaction of light with matter can do two things

Photo physical changes

Fluorescence, phosphorescence

Photo induced chemical reactions

Example of biochemical reactions: Photosynthesis, vision, DNA damage



Ionization

$$O + h\nu \rightarrow O^+ + e^-$$

Dissociation

$$N_2 + h\nu \rightarrow N + N$$

Quantum yield of photobiological processes

$$A + h\nu \rightarrow A^*$$

What's next?

- Fluorescence
- Internal conversion but no fluorescence
- Intersystem crossing
- Phosphorescence
- Photo chemical reactions

Not all absorbed photons lead to emission but there are different events

Primary quantum yield provides a quantitative measure of a specific process that happen

$$\text{Primary Quantum yield, } \phi = \frac{\text{No of events}}{\text{No of absorbed photons}}$$

$\phi = 1 \implies$ all molecules that absorbed a photon undergone an event

$\phi = 0 \implies$ all the excitation energy is lost

Primary quantum yield is ≤ 1 but 'overall' quantum yield may be not

There can be situations where absorption of single photon may lead to reaction of multiple molecules

$$HI + h\nu \longrightarrow H + I$$

$$H + HI \longrightarrow H_2 + I$$

Here, one photon allows multiple reactants to react.

$$I + I + M \longrightarrow I_2 + M$$

We define the 'overall quantum yield', Φ of a process

Here one photon leads to destruction of 2 reactant (HI) molecules, therefore, $\Phi = 2$

Quantum yield to rates

$$\textbf{Quantum yield, } \phi = \frac{\text{No of events}}{\text{No of absorbed photons}} = \frac{\text{No of events per unit time}}{\text{No of absorbed photons per unit time}}$$

$$\textbf{Therefore, } \phi = \frac{\text{Rate of photobiological events}}{\text{Rate of absorption of light}} = \frac{\text{Rate}}{\mathrm{I_{abs}}}$$

A given excited molecule may decay is many ways. So, there are many different quantum yields for each process. Sum of all those must be equal to 1.

So we can write $\sum_i \phi_i = \sum_i \frac{\mathrm{Rate_i}}{I_{abs}} = 1$

Here, i = fluorescence, IC, ISC, photochemical reactions etc.

Kinetics for fluorescence

Possible ways of deactivation of an excited singlet state

Absorption: $S + h\nu_i \longrightarrow S^*$ $\quad v_{abs} = I_{abs}$

Fluorescence: $S^* \longrightarrow S + h\nu_f$ $\quad v_f = k_f[S^*]$

Intersystem crossing: $S^* \longrightarrow T^*$ $\quad v_{ISC} = k_{ISC}[S^*]$

Internal conversion: $S^* \longrightarrow S$ $\quad v_{IC} = k_{IC}[S^*]$

Here we assume all deactivations follow first order kinetics

Now, $\dfrac{d[S^*]}{dt} = I_{abs} - k_f[S^*] - k_{IC}[S^*] - k_{ISC}[S^*]$

Experiments are done with very low [S], so if we apply a continuous radiation, [S*] will remain low and almost constant

So, steady-state approximation gives $I_{abs} = (k_f + k_{IC} + k_{ISC})[S^*]$

Finally, the lifetime of fluorescence

The primary quantum yield of fluorescence is given by

$$\phi_f = \frac{Rate\ of\ fluorescence}{I_{abs}} = \frac{k_f[S^*]}{(k_f + k_{ISC} + k_{IC})[S^*]}$$

Finally, $$\phi_f = \frac{k_f}{k_f + k_{ISC} + k_{IC}}$$

Now if we turn off light, [S*] will only decay following first order kinetics. This gives us

$$[S^*]_t = [S^*]_0 e^{-t/\tau_0}$$

Where, τ_0 is the fluorescence lifetime, defined as

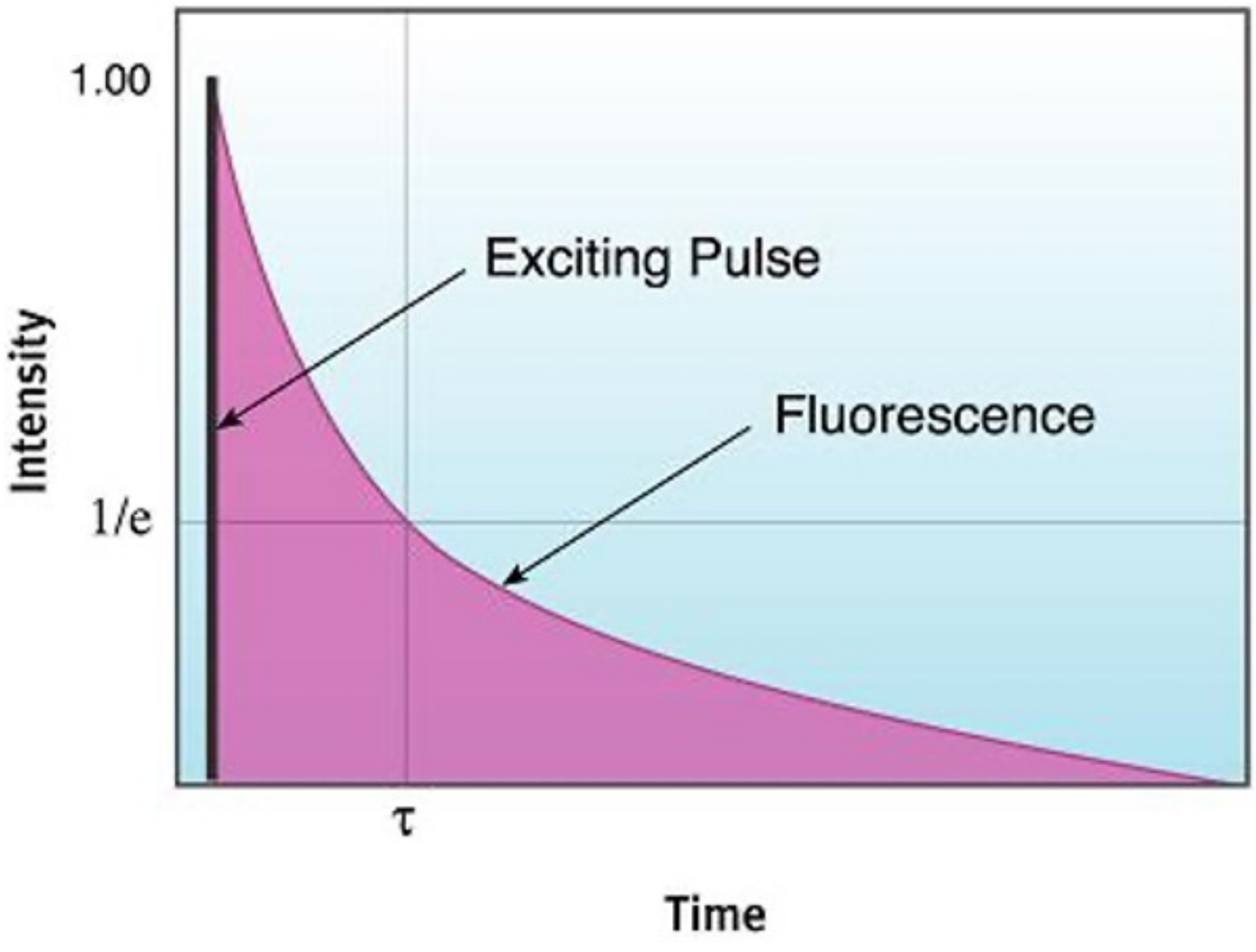
$$\tau_0 = \frac{1}{k_f + k_{ISC} + k_{IC}}$$

Final form of τ_0 becomes

$$\tau_0 = \frac{1}{k_f + k_{ISC} + k_{IC}} = \left(\frac{k_f}{k_f + k_{ISC} + k_{IC}}\right) \times \frac{1}{k_f} = \frac{\phi_f}{k_f}$$

Measuring the lifetime of fluorescence directly

- The fluorescence lifetime can be measured with a pulsed laser technique.
- First, the sample is excited with a short light pulse from a laser using a wavelength at which S absorbs strongly.
- Then, the exponential decay of the fluorescence intensity after the pulse is monitored.



Lifetimes provide idea about type of photochemical reactions

Absorption time-scale $\sim 10^{-16} - 10^{-15}\ s$

Very fast photochemical reactions, E.g. vision and photosynthesis

Life time of fluorescence $\sim 10^{-12} - 10^{-6}\ s$

Wide variety of photochemical reactions involving large number of collisions of changes in molecular geometry, E.g. photosensitization of molecular oxygen

Life time of phosphorescence $\sim 10^{-6} - 10^{-1}\ s$

Quenching of fluorescence

Fluorescence quenching is the non-radiative removal of the excitation energy from a fluorescent molecule and the elimination of its fluorescence.

Quenching can be a desired process if focusing on energy transfer or undesired side reaction decreasing the quantum yield of fluorescence

Regular deactivation channels for fluorescence

Absorption: $S + h\nu_i \longrightarrow S^*$ $\quad v_{abs} = I_{abs}$

Fluorescence: $S^* \longrightarrow S + h\nu_f$ $\quad v_f = k_f[S^*]$

Intersystem crossing: $S^* \longrightarrow T^*$ $\quad v_{ISC} = k_{ISC}[S^*]$

Internal conversion: $S^* \longrightarrow S$ $\quad v_{IC} = k_{IC}[S^*]$

The addition of a quencher, Q, opens an additional channel for deactivation of S*:

Quenching: $S^* + Q \longrightarrow S + Q$ $\quad$ *Rate of quenching* $= k_Q[Q][S^*]$