6. Fluorescence from a Single Molecule

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10 punten

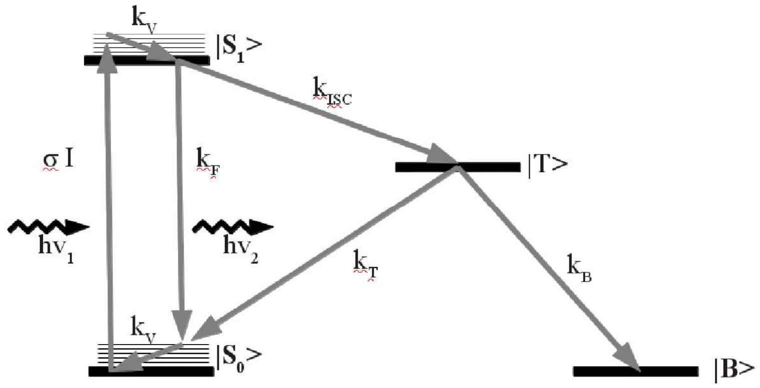

Figure 6.1: Simplified Jabłoński diagram of a fluorescent molecule. A photon $h\nu_1$ can excite the molecule from its ground state $|S_0\rangle$ to a excited state $|S_1\rangle$. From there, the molecule can emit a photon with lower energy $h\nu_2$ and go back to the ground state, or transition through a triplet (dark) state $|T\rangle$ back to the ground state, or bleach to become a photo product $|B\rangle$. When asked for a numerical answer, use the following rate constants: radiative $k_{\mathrm{F}} = 3 \times 10^8\,\mathrm{s}^{-1}$; non-radiative $k_{\mathrm{V}} = 1 \times 10^{12}\,\mathrm{s}^{-1}$, $k_{\mathrm{ISC}} = 1 \times 10^7\,\mathrm{s}^{-1}$, $k_{\mathrm{T}} = 2 \times 10^5\,\mathrm{s}^{-1}$, $k_{\mathrm{bl}} = 1 \times 10^4\,\mathrm{s}^{-1}$.

1 Find an analytical expression for the measured fluorescence life time τ_{F}, that is the life time of the excited state $|S_1\rangle$ after absorption of a photon $h\nu_1$. From the general expression, make the assumption $k_{\mathrm{V}} \gg k_{\mathrm{F}}, k_{\mathrm{ISC}}$. How does this effective fluorescence life time τ_{F}differ from $(k_{\mathrm{F}})^{-1}$?

2 Ignoring for the moment the irreversible bleaching, what is the expected time constant for one excitation emission cycle τ_{cycle}? What does this mean for the number of photons a molecule can emit per unit time? You may use the approximation $k_{\mathrm{ISC}} \gg k_{\mathrm{T}}$ in your calculations.

3 Find the excitation intensity (expressed in Wcm^{-2}) needed for a photon emission(!) rate of $5 \times 10^4\,\mathrm{s}^{-1}$ from a single molecule. To find the absorption cross section σ, you may assume that the condition are such that the Beer-Lambert law is valid and that the molar extinction coefficient of the fluorophore is $\epsilon = 80\,000\,\mathrm{M}^{-1}\,\mathrm{cm}^{-1}$ at the excitation wavelength $\lambda_{\mathrm{exc}} = 488\,\mathrm{nm}$.