Luminescence methods

Methods of biophysical chemistry - seminar

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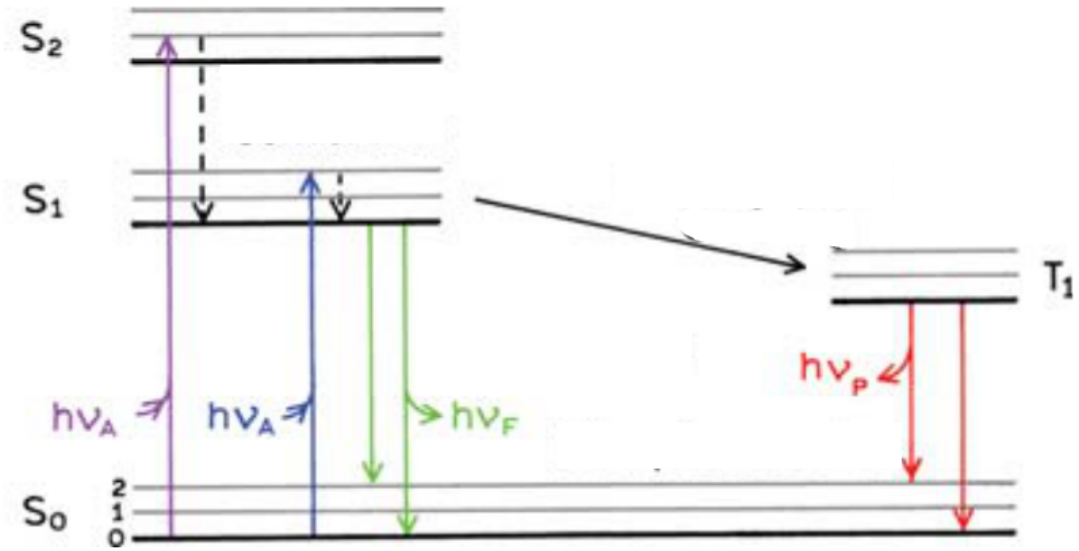
November 13, 2019

Energetic digram

Fill in attached diagram and compare the phenomena according to parameters indicated in a table:



Phenomenon	time regime	k vs. k_{vib}	order λ_{max}
Absorption			
Fluorescence			
Phosphorescence			

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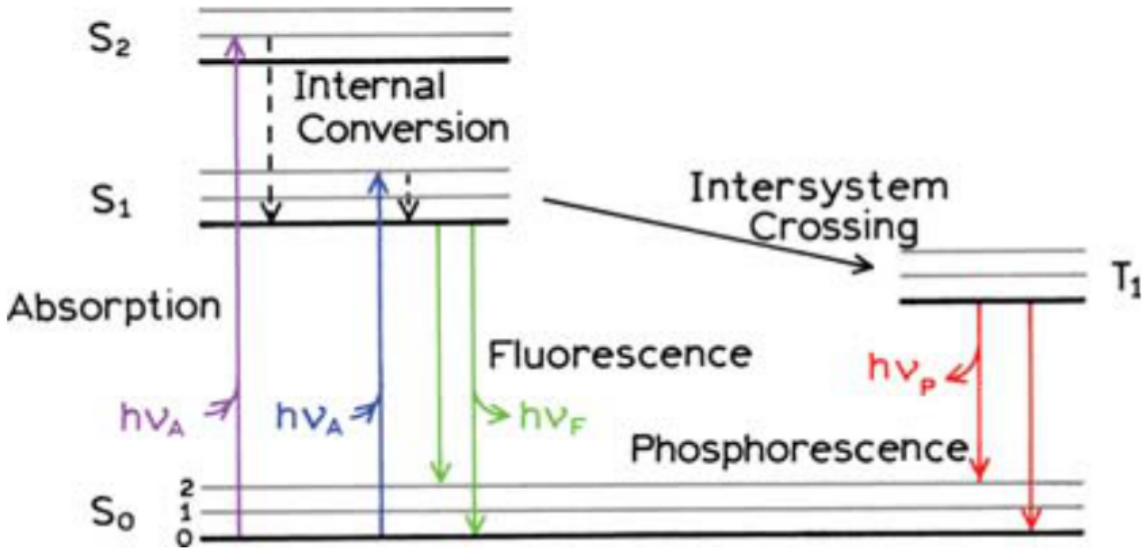

Phenomenon	time regime	k vs. k_{vib}	order λ_{max}
Absorption	10^{-15}s	$>$	1
Fluorescence	10^{-9}s	$<$	2
Phosphorescence	10^{0}s	$<$	3

Starting problems: Are the following statements true or false. Explain your decision.

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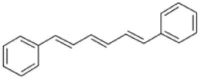
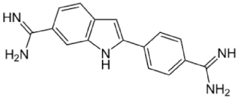
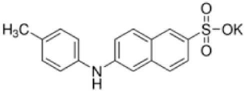
8. The measurement of fluorescence anisotropy employs circularly polarized excitation radiation

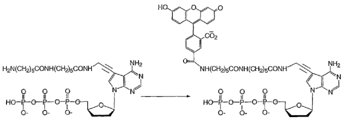
Exercise 1

Assign displayed fluorescence probes to corresponding abbreviation and biochemical application









DAPI, ddATP-Dye, DPH, TNS

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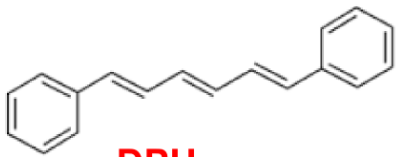
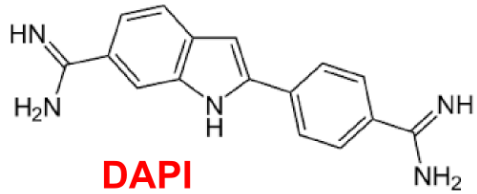
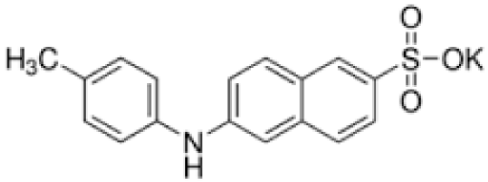


DPH
biomembranes

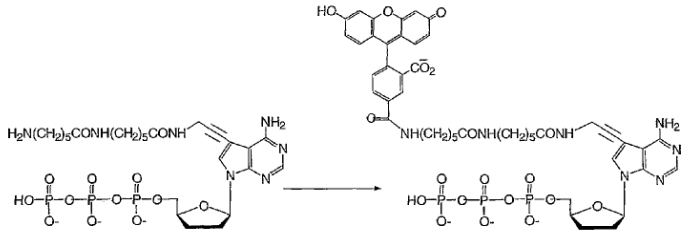


DAPI
DNA binders

1



TNS
probing proteins



ddATP-dye
nucleic acid sequencing

DAPI, ddATP-Dye, DPH, TNS

Exercise 2: Fluorescence methods

Assign the appropriate methods exploiting fluorescence techniques to following tasks:

A) Determination of hydrodynamic radius of a protein.
B) DNA hybridization.
C) Localisation of Trp residue (on surface or inside a protein).
D) Portion of unsaturated phospholipids in biomembrane.
E) Determination of K_A of eosin dimerisation.

Correlation time of fluorescence label, fluorescence anisotropy of DPH, Stokes shift, emission of excimer, FRET

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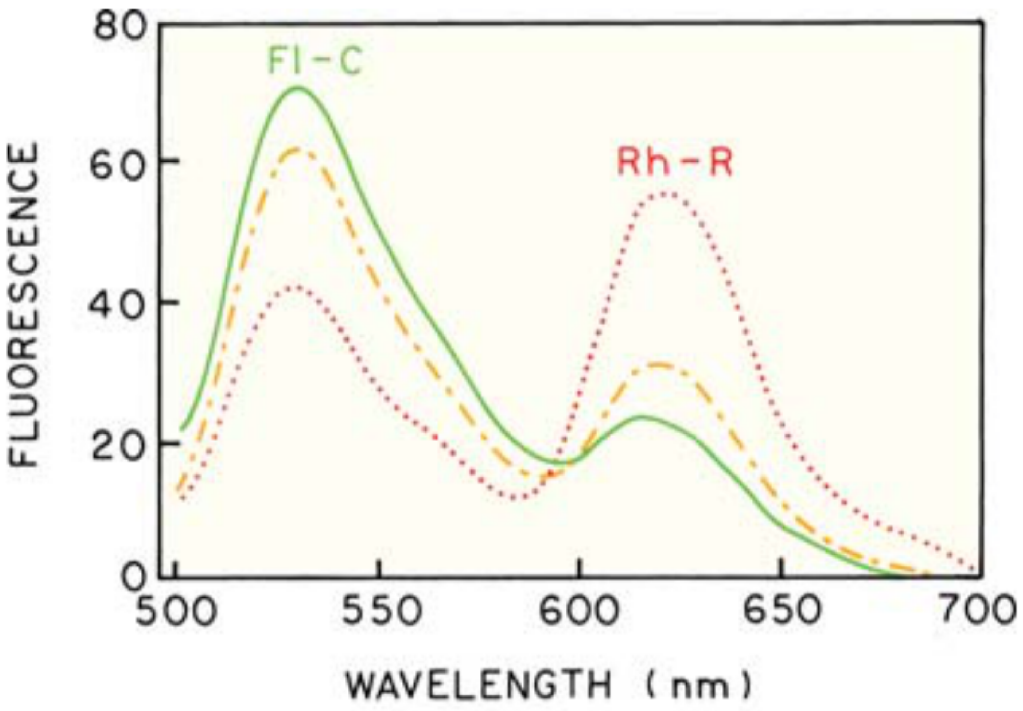
Assign the appropriate methods exploiting fluorescence techniques to following tasks:

A) Determination of hydrodynamic radius of a protein. Correlation time of fluorescence label
B) DNA hybridization. FRET
C) Localisation of Trp residue (on surface or inside a protein). fluorescence decay, Stokes shift
D) Portion of unsaturated phospholipids in biomembrane. viscosity-anisotropy of DPH
E) Determination of K_A of eosin dimerisation. emission of excimer

Correlation time of fluorescence label, fluorescence anisotropy of DPH, Stokes shift, emission of excimer, FRET

Exercise 3: FRET

Try to interpret following fluorescence experiment carried out on complex of proteinkinase consisting of catalytic (C) and regulative (R) subunit. Both parts are labelled with probes: unit C with fluorescein (Fl) and unit R with rhodamine (Rh). In native form of R_2C_2 the FRET can be detected. Identify the direction of transition and explain the effect of cAMP and PKI inhibitor added to the studied sample.



R_2C_2

R_2C_2 + cAMP

R_2C_2 + PKI

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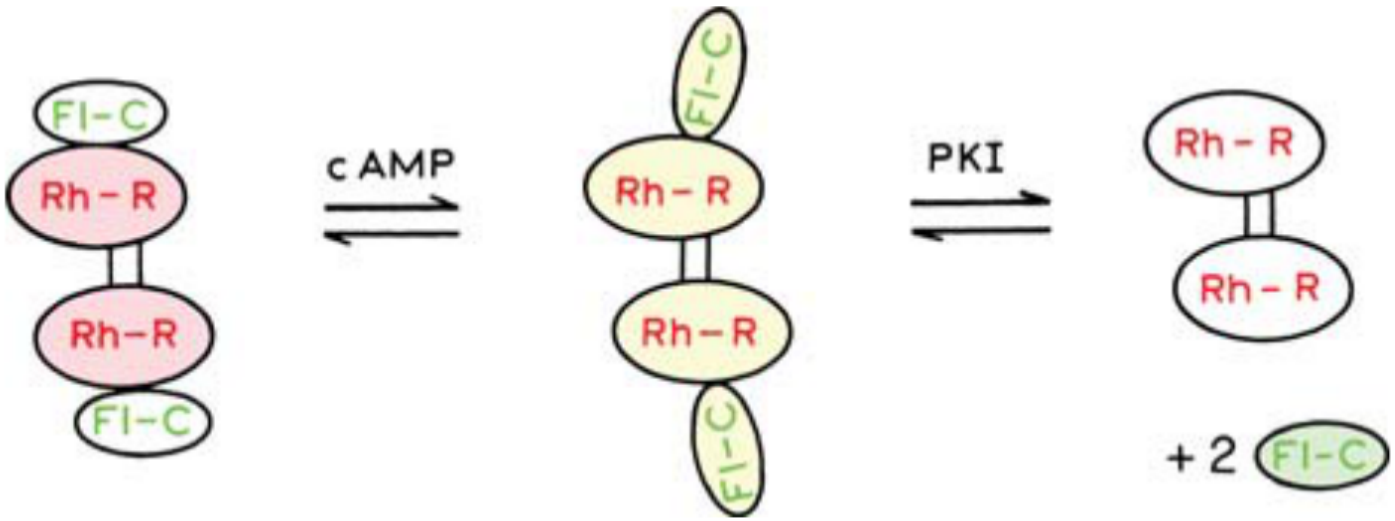

Exercise 4: Kinetic parameters of fluorescence

Eosin fluorophor is characterized by quantum yield 0.65 and fluorescence life time of 3.1 ns. Calculate the life time of radiative, non-radiative transition and intrinsic life time of fluorescence.

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Solution

$\Phi = \frac{\Gamma}{\Gamma + k_{nr}}$, $\tau = \frac{1}{\Gamma + k_{nr}} \rightarrow \Gamma = \frac{\Phi}{\tau}$

Rate constants:

$\mathbf{\Gamma} = \frac{0.65}{3.1} = 0.21\ \text{ns}^{-1}$, $\mathbf{k_{nr}} = \frac{1}{\tau} - \Gamma = \frac{1}{3.1} - 0.21 = 0.11\ \text{ns}^{-1}$

Exercise 5: Perrin equation - depolarisation of emitted signal

Based on assumption of exponential decay of intensity $I(t)$ and anisotropy $r(t)$ of fluorescnce signal derive relation between anisotropy r, life time τ and correlation time θ. Use the definition of time-weighted average of anisotropy r as a starting point:

$$r = \frac{\int_0^\infty r(t)I(t)\mathrm{d}t}{\int_0^\infty I(t)\mathrm{d}t}$$

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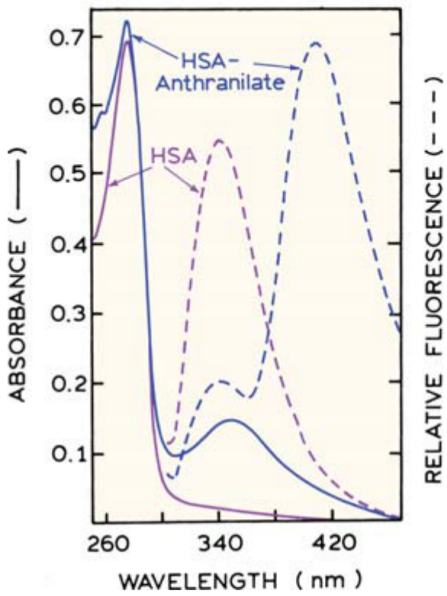
Solution

$I = I_0 \mathrm{e}^{-\frac{t}{\tau}}$, $r = r_0 \mathrm{e}^{-\frac{t}{\theta}}$

$$r = \frac{I_0 r_0 \int_0^\infty \mathrm{e}^{-t\left(\frac{1}{\tau}+\frac{1}{\theta}\right)}\mathrm{d}t}{I_0 \int_0^\infty \mathrm{e}^{-\frac{t}{\tau}}\mathrm{d}t} = \frac{r_0\left(\frac{1}{\tau}+\frac{1}{\theta}\right)^{-1}}{\tau} = \frac{r_0}{1+\frac{\tau}{\theta}}$$

Exercise 6: FRET

The protein human serum albumin (HSA) has a single tryptophan residue at position 214. HSA was labelled with an anthraniloyl group placed covalently on cysteine-34. Emission spectra of the labelled and unlabelled HSA are shown in attached figure. The Förster distance for Trp to anthraniloyl transfer is 30.3Å. Use the emission spectra in the attached figure to calculate the Trp to anthraniloyl distance. The rate constant of RET can be estimated using formula: $k_{RET} = \Gamma \left(\frac{R_0}{r}\right)^6$.



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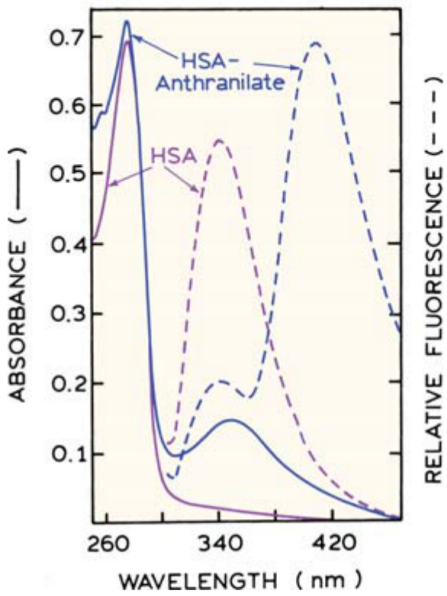


Řešení

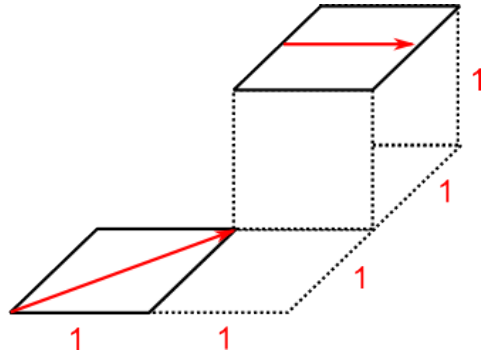
$$\Phi = \frac{\Gamma}{\Gamma + k_{RET}} = \frac{\Gamma}{\Gamma + \Gamma\left(\frac{R_0}{r}\right)^6} = \frac{r^6}{R_0^6 + r^6}$$

Quantum yield Φ at λ 340 nm: emission of albumin with acceptor/emission of free form=0.2/0.55

$$\Phi = 0.364 = \frac{r^6}{30.3^6 + r^6} \Rightarrow r = \frac{30.3}{1.75^{\frac{1}{6}}} = 27.6\text{Å}$$

Exercise 6: Dipolar interaction - orientation dependence

Efficiency of resonance transfer depends beside the spectral overlap and spatial distance between donor and acceptor on mutual orientation of transition moments. These moments interact as two dipols:
$\mu_A.\mu_B - 3(\mu_A.\mathbf{r})(\mu_B.\mathbf{r})$
Calculate the value of orientation factor κ^2 for attached model.



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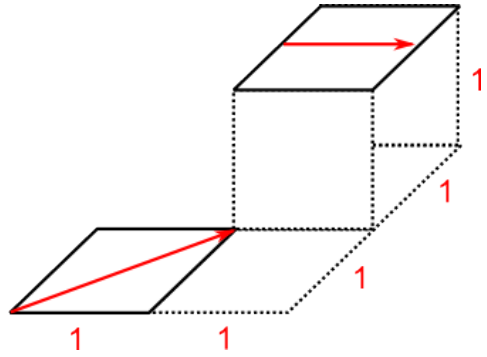

Solution

$$\kappa^2 = \left(-\frac{1}{\sqrt{2}}\right)^2$$

Further reading

Joseph R. Lakowicz: **Principles of Fluorescence Spectroscopy**
Jihad Rene Albani: **Principles and Applications of Fluorescence Spectroscopy**
P. Atkins, J. de Paula: **Physical Chemistry**