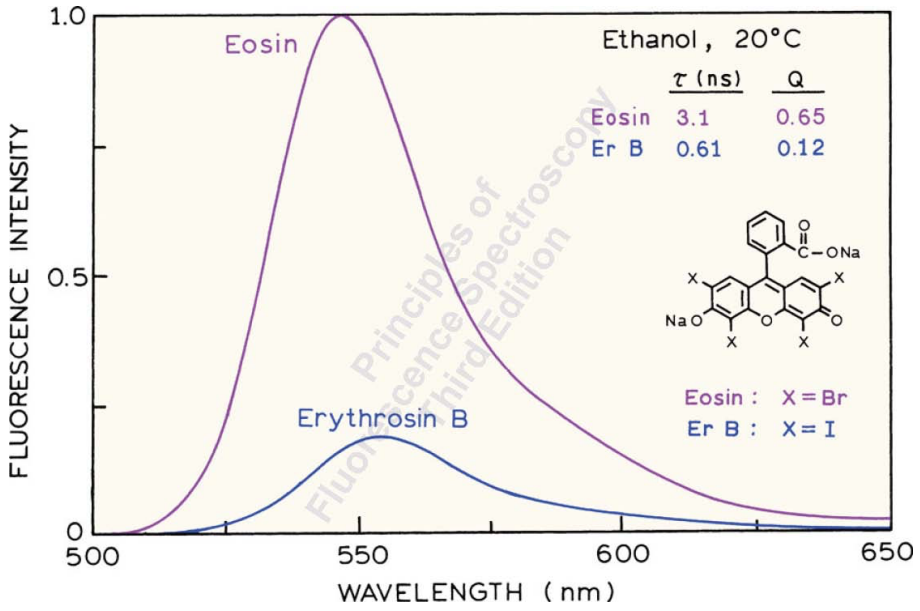
BT301 Tutorial 6 (7 Nov 2024)

1. Tryptophan and tyrosine amino acid possess the following:

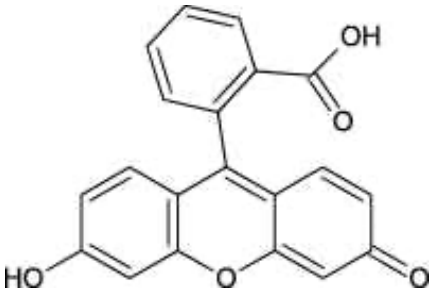
fluorophore	ε_{max} (M^{-1} cm^{-1}) @295 nm	ϕ_F
Trp	5600	0.20
Tyr	1300	0.14

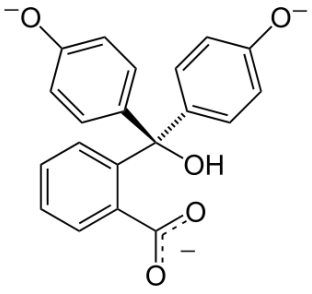
If excited at 295 nm, other conditions being identical, identify which amino acid will emit more fluorescence and by what factor?

2. Calculate the intrinsic lifetime (τ_0), k_r and k_{nr} for Eosin and Erythrosin B from the data below:



3. Given below are the structures of *Fluorescein* (left) and *Phenolphthalein* (right). Why is fluorescein more fluorescent at high pH? Explain.





4. A student varies the concentration of a fluorophore and measures its steady state fluorescence intensity. He observes that at low fluorophore concentrations, fluorescence intensity increases linearly with concentration while at high concentrations fluorescence intensity is sharply reduced deviating from linearity. Explain why?

5. A protein displays increased tryptophan fluorescence when it is exposed to solvent in the denatured state (pH 7, 6 M Guanidine.HCl) compared to native state (pH 7) when it is buried inside the protein interior. This is contrary to general observations when trp fluorescence quantum yield drops significantly when exposed to solvent (water) in comparison to non-polar protein interior. What can you infer from this observation?

6. Using the Smoluchowski equation below, calculate the diffusion-controlled rate constant for the quenching of trp by oxygen assuming a collision radius of 5 Å. Diffusion coefficients are: D_{oxygen} (298 K) = 2.5 x 10^{-5} cm^2/s; D_{trp} (298K) = 0.66 x 10^{-5} cm^2/s;

$$k_0 = \frac{4\pi N}{1000}(D_f + D_q)(R_f + R_q)$$

Where N is Avogadro number, R stands for collision radius

7. Can one molecule undergo FRET with another identical molecule of its own. So here we are talking about FRET with Donor and Acceptor being the same molecular species.

8. How is the steady state fluorescence intensity related to fluorescence intensity decay parameters like lifetime(s) and amplitude(s)?

9. In TCSPC, we keep the occurrence of STOP pulse per every START pulse deliberately low to avoid pile up problem. This implies using low concentration of fluorophore in sample which makes data acquisition extremely slow. How can we TRICK the system and avoid this problem.

10. If a molecule undergoes both dynamic and static fluorescence quenching, explain how you will plot your data to estimate the values of K_D and K_S.

11. Can one measure fluorescence emission from a SINGLE MOLECULE? If yes, explain under what conditions it can be possible? If no, explain why not?

12. Assume you have isolated a protein that contains a single-tryptophan residue, and binds dinitrophenol (DNP) in the active site. The absorption spectrum of DNP overlaps with the emission spectrum of the tryptophan residue. Assume R_0 = 50 Å and that DNP is not fluorescent. The fluorescence intensities of the tryptophan residue are 20.5 and 4.1 in the absence and presence of DNP, respectively, after correction for the inner filter effects due to the DNP absorption.

a. What is the transfer efficiency?
b. Assume that the unquenched lifetime is 5 ns. What is the expected lifetime in the presence of DNP?
c. What is the transfer rate?
d. What is the distance between the tryptophan and the DNP?
e. Assume that the solution conditions change so that the distance between the tryptophan and the DNP is 20 Å. What is the expected intensity for the tryptophan fluorescence?