

Date: May 04, 2024

Time: 2 – 5 P.M.

Maximum marks: 50

Name: \_\_\_\_\_

Roll N. \_\_\_\_\_

**Instructions**

- 1. Don't answer more than one question on one page. Start each question on a fresh page.**
- Write your name and Roll No. on question paper as well as on the answer sheet.
- The question paper carries 11 questions that span 2 pages.
- All questions in part A (8 questions) are compulsory.
- Attempt any two questions from part B (From Q. Nos. 9, 10, 11). If more than two questions are attempted, only first two will be evaluated.

**Part A: Attempt all questions**

- Which of the following DNA sequencing methods is(are) 'sequencing by synthesis' method(s)? Write all correct answers. **{2 marks}**
  - Sanger Sequencing
  - Maxam-Gilbert Sequencing
  - Next-generation sequencing
- Why are lanthanides unsuitable for measuring luminescence anisotropy? **{2 mark}**
- A lanthanide luminophore is shown on the right (Fig. 1). **{1x3 = 3 marks}**
  - What is the role of the moiety enclosed in the rectangle?
  - What is the role of the functional groups enclosed in the oval?
  - What is the role of the  $\text{Eu}^{3+}$ ?
- Luminescence decay profiles of a typical organic fluorophore, a lanthanide-based luminophore, and a metal-ligand complex are shown on the right (Fig. 2). Assign the lifetimes to these three luminophores. (Each incorrect answer invites -1 mark.) **{3 marks}**
- (a) What are quantum dots? **{1 mark}**  
(b) How do they become luminescent compared to the parent bulk material? **{3 marks}**  
(c) Name any one advantage of quantum dots over conventional fluorophores. **{1 mark}**
- The data shown in Fig. 3 are obtained from fluorescence correlation spectroscopy experiments, and the traces shown are the autocorrelation functions of two fluorescently-labelled globular macromolecules (labelled as A and B).  
  - What are the vertical and horizontal axes in this data? **{2 marks}**
  - What can you conclude about the relative sizes of the molecules? How did you reach your conclusion from this data? **{1 + 3 = 4 marks}**

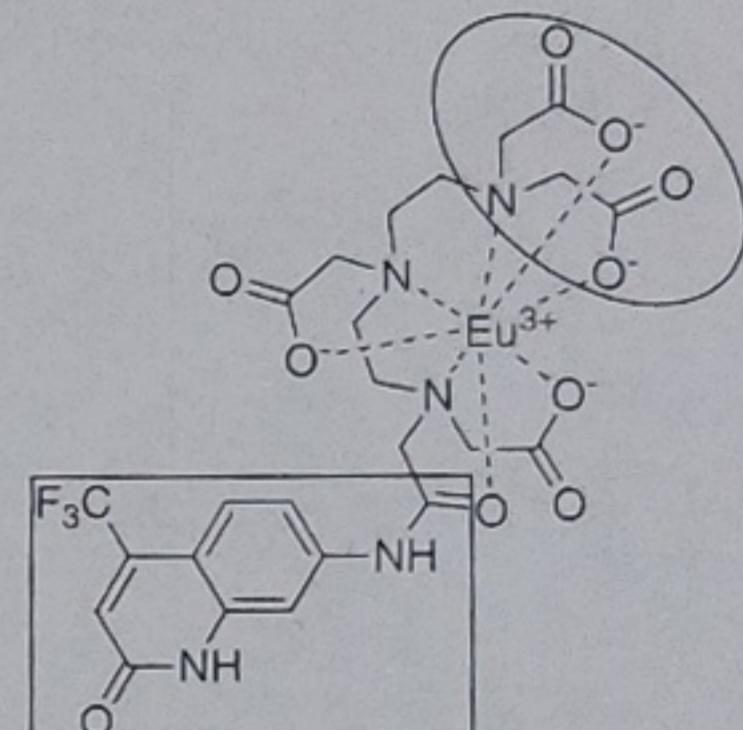


Fig. 1

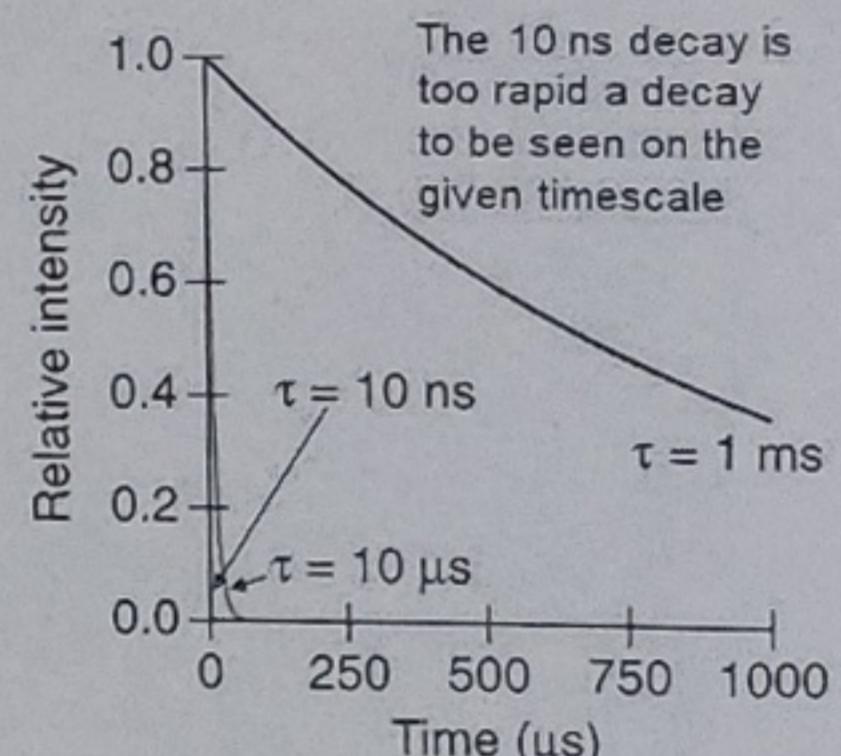


Fig. 2

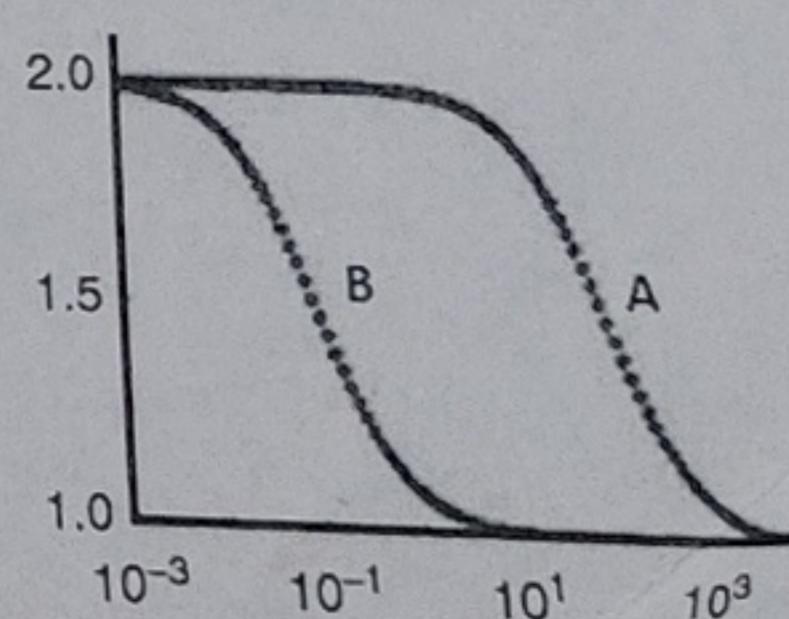


Fig. 3

7. A fluorescence-based phosphate sensor is shown in Fig. 4. Binding of phosphate affects the quantum yield and fluorescence lifetime of the fluorophore. Analyse the binding reaction shown below and answer the following questions.

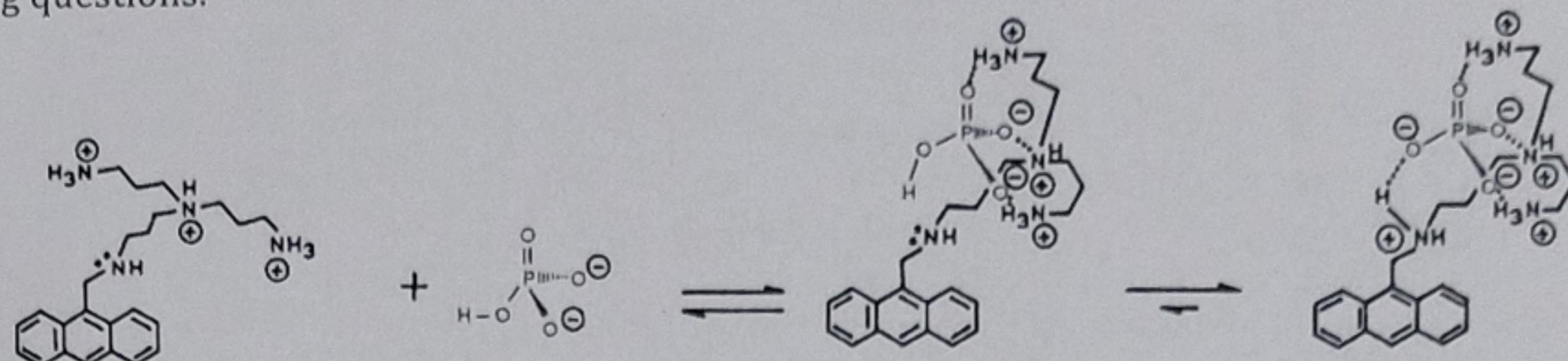


Fig. 4

- Which of the three species {1, 2, or 3} displays highest quantum yield. {1 mark}
- Explain the mechanism underlying the sensing i.e. the mechanism causing change in the quantum yield and fluorescence lifetime. {5 marks}

8. On the graph-sheet provided, draw the following diagram (Fig. 5) with the correct dimensions.

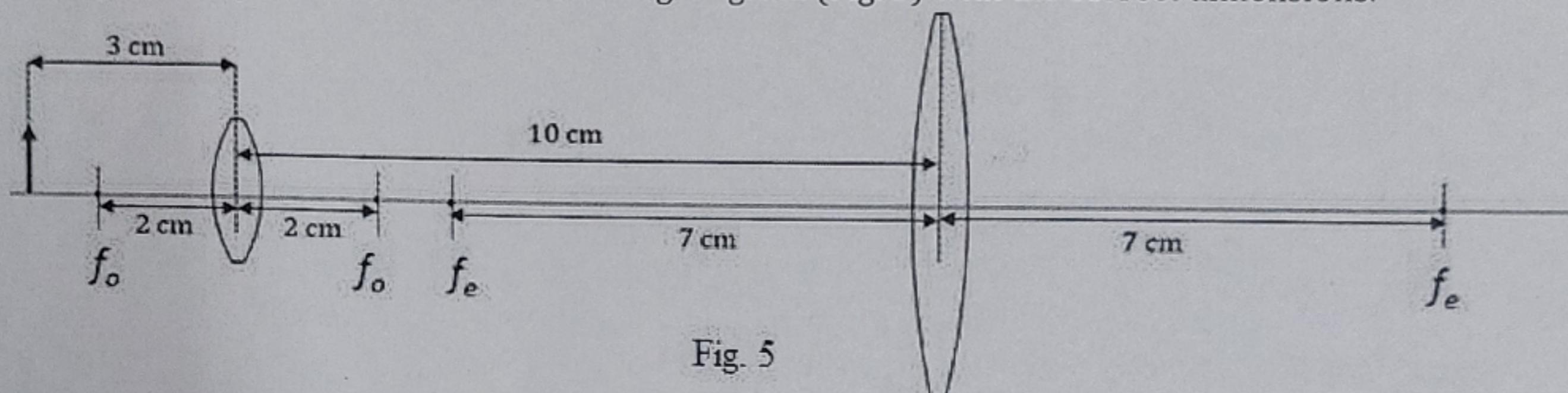


Fig. 5

- Draw a neat ray diagram to show the final image formation. {5 marks}
- What kind of final image is formed? {1 mark}
- What is the final magnification achieved as per your ray diagram? {1 mark}

-----End of part A-----

**Part B: Attempt any two questions from Q. Nos. 9, 10, 11.**

9. The synthetic dNTPs (deoxynucleotide triphosphates), wherein each nucleotide is labeled with a different fluorophore, are used in next-generation sequencing. An example of such a synthetic dNTP is shown below in Fig. 6.

- Identify the functions of the labeled moieties (enclosed by the rectangles and an oval). {2 marks}
- Using this information, briefly explain how such dNTPs allow massive parallel sequencing. (No need of mentioning library preparation. Assume that the library with different clusters of single stranded DNA molecules is already prepared on the flow cell. Just write down the steps after that sequentially). {6 marks}

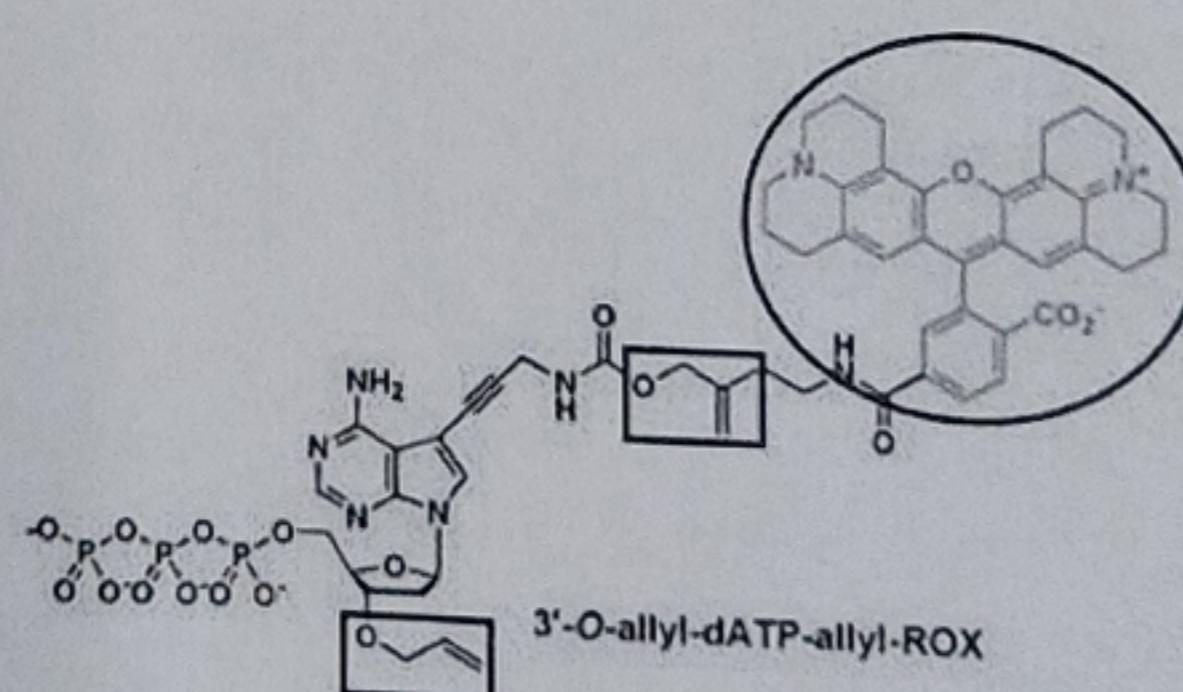


Fig. 6

10. Explain the principle of either STED (Stimulated emission depletion) or STORM (Stochastic Optical Reconstruction Microscopy). {8 marks}

11. Explain FRAP (Fluorescence recovery after photobleaching) experiment using a suitable diagram showing different phases of the experiment. {8 marks}