

Topics for acquiring ECTS credits

Note:

Please choose one of the assignments below and solve it. Please send your report to Singlemolecule@wet.kuleuven.be before **September 15th 2010**.

If handled correctly, you will receive a certificate early in October 2010.

Fluorescence microscopy (Prof. M. Sauer)

Briefly describe and compare the principle working mechanisms of STED, PALM, and dSTORM focusing on similarities, strengths, limitations, and differences? (2-3 pages)

Solid-state nanopores (Dr. R. E. Gyurcsanyi)

1. Propose and discuss three independent methods to assess the diameter of nanopores in single and multichannel solid-state membranes within the range 2-50 nm.
2. Design a selective nanopore sensing method with integrated microfluidics and detailed protocol to detect C-reactive protein (CRP). CRP is a 224 residue protein with a monomer molecular mass of approximately 25 kDa and pI 6.4, which are noncovalently associated into pentameric structure. CRP is a non-specific marker of inflammation with healthy levels below 5 mg/ml in blood serum. However in pathology has a range of ca. 0.05-500 mg/L.
3. From the solid-state nanopore fabrication technologies discussed which are those that enable large scale fabrication of nanopore based biosensors? Justify.

Protein dynamics (Dr. E. Lemke)

1. How can a mixture of an oxidant and a reductant increase photostability of fluorophores in the absence of oxygen.

- Tip: Start with the work from Vogelsang J, Angew Chem Int Ed Engl. 2008

2. For which smFRET experiments do you prefer to use a diffusion format vs immobilization of single molecules.

FRET heterogeneity analysis problem set (S. Holden)

1. Problem

Here you will derive an expression for the width of a FRET distribution for the simplest case of constant photon count for a static molecule. For simplicity, we imagine that we are lucky enough to have a perfect experimental apparatus which can directly measure the true FRET. Therefore, for our static molecule,

$$\epsilon = \frac{D}{D + A} = \frac{1}{1 + \left(\frac{R}{R_0}\right)^6}, \quad (1)$$

where ϵ is the mean FRET; D and A are the mean donor and acceptor photon counts; R is the donor-acceptor separation; and R_0 is the characteristic distance of the donor-acceptor pair, called the Förster radius.

For a static molecule, the *observed* donor and acceptor photon counts, d, a , may be treated as independent Poisson processes, with mean photon counts D, A given by:

$$D = N(1 - \epsilon), \quad A = N\epsilon, \quad (2)$$

where N is the mean total photon count, $N = D + A$, and ϵ is the mean FRET. The variance of the observed photon counts d, a is given by

$$\sigma_d^2 = D, \quad \sigma_a^2 = A, \quad (3)$$

(a) The mean FRET ϵ and mean photon counts D, A are related by Eq. 1. Show that:

i.

$$\frac{\partial \epsilon}{\partial D} = \epsilon / (D + A) \quad (4)$$

ii.

$$\frac{\partial \epsilon}{\partial A} = (1 - \epsilon) / (D + A) \quad (5)$$

(b) The width of the FRET distribution σ_E may be obtained using the formula for propagation of error of independent variables. For $f(a, b)$,

$$\sigma_f^2 = \left(\frac{\partial f}{\partial a}\right)^2 \sigma_a^2 + \left(\frac{\partial f}{\partial b}\right)^2 \sigma_b^2 \quad (6)$$

Apply Eq. 6 to Eq. 1 to show that

$$\sigma_E = \sqrt{\frac{\epsilon(1 - \epsilon)}{N}} \quad (7)$$

(c) Sketch σ_E as a function of i) ϵ and ii) N . We carry out an experiment to study a conformational change of size ΔR . What values of ϵ and N should we choose to maximise the resolution of our experiment?

Hint: to understand how to choose a FRET value ϵ to maximise distance resolution, consider the relationship between ϵ and R in Eq. 1.

2. Using the papers mentioned in the literature review, write a short (500 word) essay discussing the basic principles, advantages and disadvantages of probability distribution analysis for in vitro analysis of biological systems

Advanced FRET methods (S. Uphoff)

Have a look at the recent publications by Munro et al. on conformational changes within the ribosome (reference 1) and Chung et al. on protein folding (reference 2).

- Briefly summarize the main biological questions and central findings.
- Describe the experimental setups used in these studies. Try to draw a simple schematic and label the components. You will find additional information in the references of each paper, supporting information (available online), and on manufacturer's web pages. You might also want to consult reference 3, "A practical guide to smFRET".
- What is the purpose of the oxygen scavenging system in Munro et al.? Why was this method not useful for Chung et al.?
- What additional information do Munro et al. gain from their three color FRET experiments?
- Why was it important for Chung et al. to simultaneously record fluorescence spectra and FRET?

References

1. Munro, J.B. et al. Spontaneous formation of the unlocked state of the ribosome is a multistep process. *Proceedings of the National Academy of Sciences* 107, 709 714 (2010).
2. Chung, H.S., Louis, J.M. & Eaton, W.A. Experimental determination of upper bound for transition path times in protein folding from singlemolecule photon by photon trajectories. *Proceedings of the National Academy of Sciences* 106, 11837 11844 (2009).
3. Roy, R., Hohng, S. & Ha, T. A practical guide to singlemolecule FRET. *Nat Meth* 5, 507516 (2008).