Single Molecule Biophysics Exam Project
Blok 2, January 2009

Everybody must hand in an individually written report containing answers to all questions at latest Friday January 16, 2009, at 15.00 in Lene Oddershede's mail box.

The report MUST be in paper format, electronic versions are NOT accepted. The total report containing all answers should be between 3 and 8 pages. The project is based on the following scientific papers (which can all be downloaded from the course homepage):

- *Myosin-V makes two brownian 90° rotations per 36-nm step* by Y. Komori et al., Nature Structural & Molecular Biology **14**, pages 968–973 (2007).
- *The Bacterial Condensin MukBEG compacts DNA into a repetive, stable structure* by R.B. Case et al, Science **305**, pages 222–227 (2004).
- *Retraction* by the Bustamante group, Science **307**, page 1409 (2005)

In addition, you will need the formulas for drag coefficients and torque of rotating rods near a surface. These formulas can be downloaded via a link on the single molecule biophysics course homepage.

Myosin-V, a molecular motor

These questions relate to the above mentioned paper by Komori et al. from 2007.

1. What is the biological role of Myosin-V?
2. What is the step size of Myosin-V? How does that compare to the other motors we have discussed in the SMB course (kinesin, polymerase, ϕ29)?
3. What is the maximum translocation velocity, v_{max}? How does that compare to the velocities of kinesin and polymerase?
4. At [ATP]=1 μM what is the total average dwell time? (I.e. dwell time of the long-phase 90° rotation plus dwell time of the short-phase 90° rotations)
5. Using the above found time constant for rotating an actin filament a total of 180 degrees, find the average torque exerted on the actin filament. Make the following assumptions: Length of actin filament = 2 μm, it rotates around its center. Radius of actin filament, $r =$ 1.5 nm. Height of actin filament above surface, $h = 50$ nm. Viscosity of aqueous solution, $\eta = 0.001$ Ns/m^2. Notice that in order to use the formulas from 'Polymer Mechanics' of J. Howard, the angular velocity, ω, must be given in rad/s.
6. Calculate the energy needed to make 2 x 90° rotations of the above mentioned actin filament.
7. In each step of the Myosin-V motor, one ATP is hydrolyzed. How big a fraction of the liberated energy is spent to rotate the actin filament?

8. Explain the 'Brownian rotation hand-over-hand model' proposed in this paper and why this is thought to be an advantageous method for transporting cargo in cells.

Critical reading of scientific papers

One purpose of the single molecule biophysics course has been to achieve a critical perspective on published scientific papers. This is part of the topic of the last part of this exam project. The following questions relate to the Case et al. paper from 2004 and the corresponding retraction from 2005.

9. Explain the biochemical role of Bacterial Condensin MukBEF

10. Calculate the work put into unfolding and refolding the structure using the traces shown in the right hand side of Figure 1 (A) (ignore the fact that part of the energy goes into stretching the backbone, just find the total work performed). Give the values both in units of joule and in units of $k_B T$ for T=300 K.

11. Suppose an experiment was carried out in which a structure was unfolded and re-folded using the same force loading rate, the system being switched between two equilibrium states. And suppose that the following sets of unfolding and re-folding total works (given in numerical values) were measured:

UN-FOLDING Number of counts	Work Center value $[k_B T]$	**RE-FOLDING** Number of counts	Work Center value $[k_B T]$
3	8500	6	7000
6	9000	11	7500
11	9500	14	8000
16	10000	10	8500
8	10500	5	9000
1	11000	1	9500

(Notice that these values are constructed, they are not in the material published by Case et al.).

Using this information find the Gibbs free energy difference of the unfolding process. Explain how you do, preferably illustrating your method with a plot of the unfolding/re-folding probability distributions.

12. What are the main observations and conclusions stated in the paper by Case et al. from 2004?

13. What went terribly wrong? What was really pulled apart in the experiment reported by Case et al.?

14. Could Case et al. have know this prior to publication? Try to find possible warning signs in the paper.

Lene Oddershede, Niels Bohr Institute, December 19, 2008.