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EXAM
TFY4335 BIONANOTECHNOLOGY

15th of December 2012. 09:00

Examination support materials:

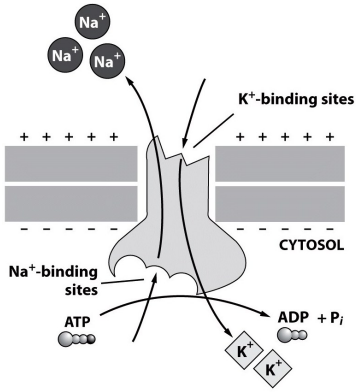
- Formula sheet - see Appendix A
- Simple calculator (according to NTNU exam regulations)
- K. Rottmann: Matematisk formelsamling (eller tilsvarende)
- Carl Angell og Bjørn Ebbe Lian: Fysiske størrelser og enheter, navn og symboler (eller tilsvarende)

Answer must be written in English or Norwegian. The maximum score for the exam is **100p**.

Question 1: Explain briefly (25p)

1. Why it is difficult to mix fluids in microfluidic devices? **(5p)**
   No turbulences, viscous flow; velocity is not changing with time and is defined everywhere in the flow; flow stops as soon as we stop pumping; fluid has no sideways velocity and moves only along pressure gradient in well defined "streams". Mixing only by diffusion which is slow at longer distances ($> 10\mu$m). Note: it is not that the viscosity is larger for fluids in small devices, viscosity is an intrinsic property of the fluid and do not depend on where the fluid is flowing. But in small devices it is dominating as the fluid inertia is very very small comparing to viscous friction.
2. What is defined by Nernst potential and how is it related to action potential? **(10p)**
   Equilibrium potential needed to maintain certain ion concentration difference (or created by concentration gradient if the ions can not move due to presence of a membrane). Membrane resting potential will contains contributions from the Nernst potential from ions which have a different concentration on two sides of the membrane (if at equilibrium different ionic species have different conductivity across the membrane, it will be ions with the highest conductivity which will set the membrane potential). Open ion channels will result in ion flow which will try to equilibrate concentration differences (and at the same time will reduce the membrane potential). Local and time limited change in membrane potential (away from resting potential, towards Nernst potential of the ionic species with highest conductivity conducted through the open ion channels) due to opening and subsequent closing of the voltage gated ion channels is the action potential

3. Ion pumps are molecular motors which use chemical energy of ATP molecules. What are the free energy "costs" connected to pumping of ions from one to the other side of a cell membrane? Figure below might be helpful. **(5p)**



Moved both against electric field (Na ions only) and concentration gradient (both); for Na/K pump K are moved with electric field but against concentration gradient; Na are moved against both. Pumping against concentration gradient is reducing the entropy of the system (chemical potential, this costs energy from ATP); against the electric field is increasing the energy contribution (also cost chemical energy from ATP).

4. What is described by term *osmotic pressure*. What is the origin of that phenomena and what is necessary for it to be observed? **(5p)**

polish: Entropy of molecules/particles dissolved will increase if the volume which they can explore is increased; entropic force; only exist if there is a membrane inpenetrable for particles separating two zones with different concentration. Osmotic Pressure is the pressure difference one would need to apply to stop the flow

Question 2: Brownian motion and particle size (25p)

Imagine that you are newly employed by a company which would like to develop an instrument to measure particle size by analysis of particles brownian motion. This instrument is based on newly developed super resolution imaging system (microscope that can take pictures of particles in solution). Your microscope has a resolution up to 10nm/pixel, but can take images of an area not larger than 1000x1000pixels.

1. Describe how to determine particle size from measured particle trajectories.

As in the lab; mean square step size $\langle \Delta^2 \rangle$ and use the distribution of step sizes to calculate diffusion constant and the particle diameter (from Einstein-Stokes equation; viscosity and temperature need to be known, one assume that the particle is perfectly spherical). Using mean square displacement $\langle x^2 \rangle$ or only λ_{1D} or λ_{2D} is not a preferred way of doing this as λ will depend on drift velocity and in case of any drift, would result in wrong value for diffusion constant (one could compensate for this by looking at many particles and looking at the distribution of $\langle x^2 \rangle$; this would need to be explained if you follow that approach).

2. Your instrument is only able to track particles in 2 lateral dimensions (xy) and not in the vertical direction (up/down, z). Particle moving along z (up or down) in the sample chamber will appear stationary in the microscope). Is this a problem which will effect your measurements? No since all spacial direction are independent and in principle it is sufficient to follow motion along one direction, even if the particle is moving in 3D.

3. Assume that, to determine particle size, one need to track its trajectory for at least 10 frames. Determine frame rate (number of images per second) which your camera must take, so you can measure particles as small as 30nm in diameter.

We need to calculate time in which a particle will travel half of the image size (using whole image size is also acceptable); assuming no drift; the same velocity in all direction. Then there is a chance that if it starts with the particle at the centre of the image, it will still be visible after 10 frames. We have a particle with $R = 15 \times 10^{-9}$m. Image size $A = 1000 \cdot 10 \times 10^{-9} = 10 \times 10^{-6}$m.

$$D = \frac{k_B T}{6\pi\eta R} = \frac{4.21 \times 10^{-21}}{6\pi \cdot 1 \times 10^{-3} \cdot 15 \times 10^{-9}} = 1.5 \times 10^{-11}\mathrm{m}^2\mathrm{s}^{-1} \quad (1)$$

$$\lambda_{1D}^2 = \langle x^2 \rangle = 2Dt = 2Dn\tau \quad (2)$$

If we find time in which a particle will on average travel a distance $A/2$, starting from the screen centre, then around 68% of the particles we follow will not disappear from the screen. If we set the frame rate to the time a particle travel A/4, then 95% of the particle will stay in the view screen (λ_{1D}^2 is the σ^2 of the distribution of how far we expect the particle to move, 68% of the paricles will not move more then 1σ, 95% of the particles will not move more then 2σ).

$$\lambda_{1D} = 5 \times 10^{-6}\mathrm{m}$$
$$\tau = \frac{\lambda^2}{2Dn}$$
$$\tau = \frac{(5 \times 10^{-6})^2}{2 \cdot 10 \cdot 1.5 \times 10^{-11}} = 0.083s$$

So the frame rate should be not less then 12 frames/sec. For

$$\lambda_{1D} = 2.5 \times 10^{-6}\mathrm{m}$$
$$\tau = 0.083/4 = 0.021$$

and the frame rate is ≈ 50 frames/sec.

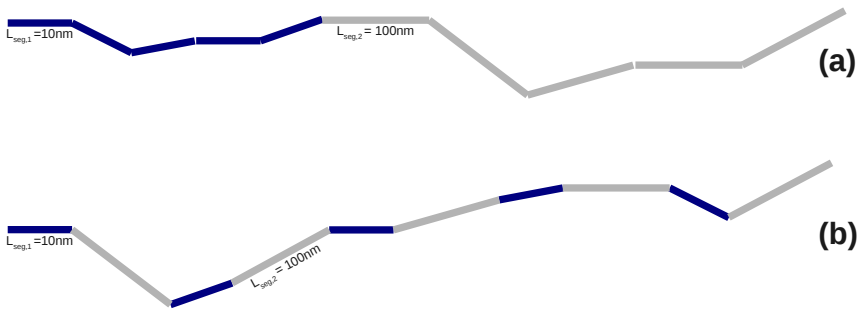
4. Could you used the same system to measure surface charge (zeta potential; $\zeta-$potential) of your particles. Explain briefly how this could be possible. Hint: elecrophoretic mobility is proportional to ζ.
   add external electric field and measure drift velocity using particle tracking system. Elecrophoretic mobility is proportional to ζ.

Question 3: Polymer chins (25p)

You are using optical tweezers to perform experiments with single polymer chain which has its ends attached to two polymer beads 1μm in diameter.

1. Describe main principles of how laser light can be used to manipulate small objects.
   High intensity laser focus to a point with very high intensity gradient. Intensity gradient is needed for crating the trapping force both for larger particles ($D \gg \lambda$ where it can be explained based on geometric optics and change of direction of the momentum vector of incoming photons) and small particle ($D \ll \lambda$), where the force is a results of interaction between induced dipole moments within the trapped object and the electric field created by the laser light
2. Your friend working at biochemistry department has made you 2 special polymers
   (a) this polymer is made of two different polymer chains connected together in a "end-to-end" fashion as shown below. Both chains have the same length, one can be described by $L_{seg,1} = 10$nm and the other by $L_{seg,2} = 100$nm

(b) this polymer contains the same chemical units, this time connected in an alternating fashion. For simplicity assume that chemical segments have the same length as L_{seg} (see Figure).



Use 1D FJC model to describe expected force-extension behaviour for polymer (a). Hint: For 2 springs connected in series $f = -k_1 x_1 = -k_2 x_2$; in parallel $f = -(k_1 + k_2)x$.

The problem is equivalent to two springs connected in series, and both "polymer springs" will experience the same force f, and the total extension will be sum of extension for two polymers. The question is perhaps not 100% precise and one can think that the two chains have the same length or the same number of segments. Both interpretations are ok.

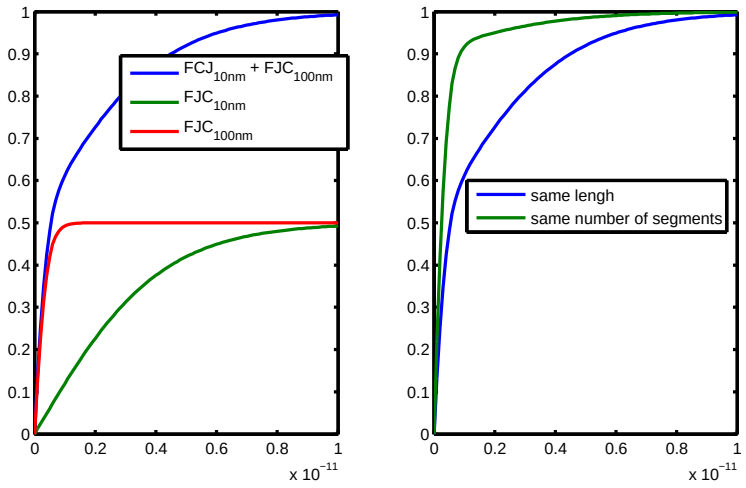
$$\begin{aligned}
\langle z_1 \rangle &= N_1 L_{seg,1} \tanh\left(f L^{(1d)}_{seg,1}/k_B T\right) \\
\langle z_2 \rangle &= N_2 L_{seg,2} \tanh\left(f L^{(1d)}_{seg,2}/k_B T\right) \\
\langle z_1 \rangle + \langle z_2 \rangle &= N_1 L_{seg,1} \tanh\left(f L^{(1d)}_{seg,1}/k_B T\right) + N_2 L_{seg,2} \tanh\left(f L^{(1d)}_{seg,2}/k_B T\right)
\end{aligned}$$

If we assume that two "sub-polymers" have the same length $L_{tot}/2$

$$\begin{aligned}
\langle z_1 \rangle + \langle z_2 \rangle &= \frac{L_{tot}}{2}\left\{\tanh\left(f L^{(1d)}_{seg,1}/k_B T\right) + \tanh\left(f L^{(1d)}_{seg,2}/k_B T\right)\right\} \\
\frac{\langle z_1 \rangle + \langle z_2 \rangle}{L_{tot}} &= \frac{1}{2}\left\{\tanh\left(f L^{(1d)}_{seg,1}/k_B T\right) + \tanh\left(f L^{(1d)}_{seg,2}/k_B T\right)\right\}
\end{aligned}$$

If we assume that two "sub-polymers" have the same number of segments

$$\begin{aligned}
\langle z_1 \rangle + \langle z_2 \rangle &= N\left\{L_{seg,1} \tanh\left(f L^{(1d)}_{seg,1}/k_B T\right) + L_{seg,2} \tanh\left(f L^{(1d)}_{seg,2}/k_B T\right)\right\} \\
\frac{\langle z_1 \rangle + \langle z_2 \rangle}{L_{tot}} &= \frac{1}{N\left(L_{seg,1} + L_{seg,2}\right)} N\left\{L_{seg,1} \tanh\left(f L^{(1d)}_{seg,1}/k_B T\right) + L_{seg,2} \tanh\left(f L^{(1d)}_{seg,2}/k_B T\right)\right\} \\
\frac{\langle z_1 \rangle + \langle z_2 \rangle}{L_{tot}} &= \frac{1}{\left(L_{seg,1} + L_{seg,2}\right)}\left\{L_{seg,1} \tanh\left(f L^{(1d)}_{seg,1}/k_B T\right) + L_{seg,2} \tanh\left(f L^{(1d)}_{seg,2}/k_B T\right)\right\}
\end{aligned}$$



What do you think will happen for polymer (b) when we try to describe its behaviour using the same model (1D FJC)? This might be helpful:

$$\langle z\rangle = k_BT\frac{\mathrm{d}}{\mathrm{d}f}\ln\left[\left(\sum_{\sigma_1=\pm1} e^{fL^{(1d)}_{seg}\sigma_1/k_BT}\right)\times \ldots\ldots\times\left(\sum_{\sigma_N=\pm1} e^{fL^{(1d)}_{seg}\sigma_N/k_BT}\right)\right]$$

$\langle z\rangle$ do not depend on the order of individual segments, so in principle this polymer chains should behave in the same manner as the polymer chain discussed above. In that case the equation can be written as

$$\langle z\rangle = k_BT\frac{\mathrm{d}}{\mathrm{d}f}\ln\left[\left(\sum_{\sigma_1=\pm1} e^{fL^{(1d)}_{seg,1}\sigma_1/k_BT}\right)\left(\sum_{\sigma_2=\pm1} e^{fL^{(1d)}_{seg,2}\sigma_2/k_BT}\right)\left(\sum_{\sigma_3=\pm1} e^{fL^{(1d)}_{seg,1}\sigma_3/k_BT}\right)\times \ldots\right]$$

$$\langle z\rangle = k_BT\frac{\mathrm{d}}{\mathrm{d}f}\ln\left[\left(\sum_{\sigma_1=\pm1} e^{fL^{(1d)}_{seg,1}\sigma_1/k_BT}\right)\times \ldots\ldots\times\left(\sum_{\sigma_{N1}=\pm1} e^{fL^{(1d)}_{seg,1}\sigma_{N1}/k_BT}\right)\times \left(\sum_{\sigma_1=\pm1} e^{fL^{(1d)}_{seg,2}\sigma_1/k_BT}\right)\times \ldots\ldots\times\left(\sum_{\sigma_{N2}=\pm1} e^{fL^{(1d)}_{seg,2}\sigma_{N2}/k_BT}\right)\right]$$

Question 4: Potential near a protein molecule (25p)

Consider a protein sphere with a radius of 18Å, and charge $Q = -10e$, in an aqueous solution of c1 = 0.05M NaCl at 25 ^{o}C. We consider the small ions as point charges and use the linear approximation to the Poisson-Boltzmann equation (Debye-Hükel equation)

1. electrical potential form a charged sphere in a salt solution is given by

$$V(r) = \frac{A}{r}e^{-r/\lambda_D}$$

where A is a constant. Find A by comparing electric field from a sphere ($E(R)$)with charge Q with the field calculated from the equation above (those have to be equal on the sphere surface)

2. What is the surface potential of the protein in units $\frac{k_BT}{e}$?

3. What is the concentration of Na^+ ions and of Cl^- ions at the surface of the protein?
4. What is the concentration of Na^+ and Cl^- ions at a distance of 3Å from the protein surface?

$$E_r(R) = \frac{Q}{4\pi\epsilon_0\epsilon R^2} \qquad E = -\frac{\mathrm{d}V}{\mathrm{d}r} \qquad \lambda_D = 0.31[\mathrm{NaCl}]^{-1/2} \quad k_BT/e = 25\mathrm{mV}$$

We first need to calculate E from the potential expression above and assume that on the sphere surface electric field from charged sphere and from potential above are equal

$$\begin{aligned}
\frac{1}{4\pi\epsilon_0\epsilon}\frac{Q}{R^2} &= -\frac{\mathrm{d}V}{\mathrm{d}r}\bigg|_{r=R} = \frac{A}{R^2}e^{-\frac{R}{\lambda_D}} + \frac{A}{R\lambda_D}e^{-\frac{R}{\lambda_D}} = \frac{A}{R^2}e^{-\frac{R}{\lambda_D}}\left(1+\frac{R}{\lambda_D}\right) \\
\frac{1}{4\pi\epsilon_0\epsilon}\frac{Q}{R^2} &= \frac{A}{R^2}e^{-\frac{R}{\lambda_D}}\left(1+\frac{R}{\lambda_D}\right) \\
A &= \frac{Q}{4\pi\epsilon_0\epsilon}\frac{1}{\left(1+\frac{R}{\lambda_D}\right)}e^{\frac{R}{\lambda_D}}
\end{aligned}$$

Now we can calculate $V(r)$

$$V(r) = \frac{A}{r}e^{-r/\lambda_D} = \frac{Q}{4\pi\epsilon_0\epsilon r}\frac{1}{\left(1+\frac{R}{\lambda_D}\right)}e^{\frac{R-r}{\lambda_D}}$$

We need the potential in the units of k_BT/e to be able to use the Boltzmann distribution to get the ion concentration. Using

$$\begin{aligned}
l_B &= \frac{e^2}{4\pi\epsilon_0\epsilon k_BT} \\
\frac{1}{4\pi\epsilon_0\epsilon} &= \frac{k_BTl_B}{e^2} \\
V(r) &= \frac{k_BTl_B}{e^2}\frac{Q}{r}\frac{1}{\left(1+\frac{R}{\lambda_D}\right)}e^{\frac{R-r}{\lambda_D}} = \frac{Ql_B}{er}\frac{e^{\frac{R-r}{\lambda_D}}}{\left(1+\frac{R}{\lambda_D}\right)}\frac{k_BT}{e}
\end{aligned}$$

and

$$\begin{aligned}
V(R = 18\text{Å}) &= -1.7\frac{k_BT}{e} \\
V(R = 18\text{Å} + 3\text{Å}) &= -1.2\frac{k_BT}{e}
\end{aligned}$$

Now we can calculate the concentrations from

$$\begin{aligned}
c_+ &= c_\infty e^{-eV(r)/k_BT} \\
c_- &= c_\infty e^{+eV(r)/k_BT}
\end{aligned}$$

Giving at R = 18Å

$$\begin{aligned}
c_+ &= 270\mathrm{mM} \\
c_- &= 9\mathrm{mM}
\end{aligned}$$

and Giving at R = 21Å

$$c_+ = 170\text{mM}$$
$$c_- = 15\text{mM}$$

This is significantly different then $c_\infty = 50\text{mM}$

Appendix A: Equation Sheet

$$k_B = 1.38 \times 10^{-23}\text{J K}^{-1} \quad (3)$$

$$e = 1.6 \times 10^{-19}\text{coul.} \quad (4)$$

$$\varepsilon_0 = 8.9 \times 10^{-12}\text{F m}^{-1} \quad (5)$$

$$\eta_{water} = 1 \times 10^{-3}\text{Pa s} \quad (6)$$

$$v_{drift} = \frac{f}{\xi} \quad (7)$$

$$\xi = 6\pi\eta R \quad (8)$$

$$\xi D = k_B T \quad (9)$$

$$\lambda_X = \sqrt{2Dt} \quad (10)$$

$$\lambda_{3D} = \sqrt{6Dt} \quad (11)$$

$$\left\langle r^2 \right\rangle = NL_{seg}^2 \quad (12)$$

$$D = \frac{1}{\tau}\int_{-\infty}^{\infty}\frac{\Delta^2}{2}\rho(\Delta)d\Delta \quad (13)$$

$$2D\tau = \left\langle \Delta^2 \right\rangle \quad (14)$$

$$\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial x^2} \quad (15)$$

$$j_s = -D\frac{\partial c}{\partial x} \quad (16)$$

$$\frac{\partial c}{\partial t} = -\frac{\partial j}{\partial x} \quad (17)$$

$$j_s = -P_s \Delta c \quad (18)$$

$$\frac{\partial c}{\partial t} = D\nabla^2 c \quad (19)$$

$$\vec{j} = -D\nabla c \quad (20)$$

$$c(\vec{r},t) = \frac{N}{(4\pi Dt)^{3/2}}e^{-\frac{r^2}{4Dt}} \quad (21)$$

$$j = D\left(-\frac{dc}{dx} + \frac{q}{k_B T}\varepsilon c\right) \quad (22)$$

$$\Delta\left[\ln c\right] = -\frac{q}{k_B T}\Delta V \quad (23)$$

$$c\left(z\right) = Ce^{\frac{-m_{\text{eff}}gz}{k_B T}} \quad (24)$$

$$j\left(r\right) = D\left(-\frac{dc}{dr} + \frac{r\omega^2 m_{\text{eff}}}{k_B T}c(r)\right) \quad (25)$$

$$c(r) = Ce^{\frac{m_{net}\omega^2 r^2}{2k_B T}} \quad (26)$$

$$v_{crit} = \frac{\eta}{\rho R} \quad (27)$$

$$f_{crit} = \frac{\eta^2}{\rho_m} \quad (28)$$

$$f_{fric} = \frac{\eta\ell^3 v}{R^2} \quad (29)$$

$$f_{inert} = \frac{\rho_m \ell^3 v^2}{R} \quad (30)$$

$$\Re = \frac{vR\rho}{\eta} \quad (31)$$

$$\frac{f}{A} = -G\frac{\Delta z}{d} \quad (32)$$

$$\frac{f}{A} = -\eta\frac{v}{d} \quad (33)$$

$$Q = \frac{\pi R^4 p}{8L\eta} \quad (34)$$

$$\frac{k_B T}{2} = \alpha\frac{\left\langle x^2 \right\rangle}{2} \quad (35)$$

$$S \equiv k_B \ln\Omega \quad (36)$$

$$T^{-1} = \left(\frac{dS}{dE}\right) \quad (37)$$

$$\Delta U = \Delta Q + \Delta W \quad (38)$$

$$\Delta S \geq \frac{\Delta Q}{T} \quad (39)$$

$$F_a \equiv E_a - TS_a \quad (40)$$

$$G_a \equiv E_a + pV_a - TS_a \quad (41)$$

$$\frac{P_1}{P_2} = e^{\frac{\Delta E}{k_B T}} \quad (42)$$

$$P_1 = \frac{1}{1+e^{-\frac{\Delta E}{k_B T}}} \quad (43)$$

$$P_2 = \frac{1}{1+e^{\frac{\Delta E}{k_B T}}} \quad (44)$$

$$\tau^{-1} = Ce^{\frac{-\Delta E^{\ddagger}}{k_B T}}\left(1+e^{\frac{-\Delta E}{k_B T}}\right) \quad (45)$$

$$\Delta F = \Delta F_0 - f\Delta z \quad (46)$$

$$Z = \sum_j e^{-E_j/k_B T} \quad (47)$$

$$p_{equil} = c_{osm}k_B T \quad (48)$$

$$c_{osm} = \varphi M c \quad (49)$$

$$\Sigma = Rp/2 \quad (50)$$

$$\ell_B \equiv \frac{e^2}{4\pi\varepsilon k_B T} \quad (51)$$

$$\overline{V}(x) = \frac{eV(x)}{k_B T} \quad (52)$$

$$c_+(x) = \frac{2\pi\ell_B\left(\frac{\sigma_q}{e}\right)^2}{\left(1+2\pi\ell_B\frac{\sigma_q}{e}x\right)^2} \quad (53)$$

$$x_0 = \left(\frac{e}{2\pi\ell_B\sigma_q}\right) \quad (54)$$

$$\frac{\mathrm{d}^2\overline{V}}{\mathrm{d}x^2} = -4\pi\ell_B c_0 e^{-\overline{V}} \quad (55)$$

$$\lambda_D = \left(8\pi\ell_B c_\infty\right)^{-\frac{1}{2}} \quad (56)$$

$$V(x) = -\frac{\sigma_q\lambda_D}{\varepsilon}e^{-\frac{x}{\lambda_D}} \quad (57)$$

$$\lambda_D = 0.31[\text{NaCl}]^{-1/2} \quad (58)$$

$$\frac{E}{A} \approx k_B T\left(\frac{\sigma}{e}\right) \quad (59)$$

$$\frac{E}{A} \approx k_B T\left(\frac{\sigma}{e}\right)^2 2\pi\lambda_D\ell_B \quad (60)$$

$$f = 2k_B T b^2 r \quad b^2 \propto \frac{1}{nl^2} \quad (61)$$

$$\left\langle z/L_{tot}\right\rangle = \tanh\left(fL_{seg}^{(1d)}/k_B T\right) \quad (62)$$

$$\left\langle z/L_{tot}\right\rangle = \coth\left(fL_{seg}/k_B T\right) - \left(fL_{seg}/k_B T\right)^{-1} \quad (63)$$

$$\left\langle z/L_{tot}\right\rangle = \frac{\sinh\alpha}{\sqrt{\sinh^2\alpha + e^{-4\gamma}}} \quad (64)$$

$$\alpha \equiv \frac{f\ell}{k_B T} \quad \alpha \equiv \frac{\Delta G}{-2k_B T} \quad (65)$$

$$j(x) = cv_{drift} - D\frac{\mathrm{d}c}{\mathrm{d}x} \quad (66)$$

$$j^{(1D)} = -MD\left(\frac{\mathrm{d}P}{\mathrm{d}x} + \frac{1}{k_B T}P\frac{\mathrm{d}U_{tot}}{\mathrm{d}x}\right) \quad (67)$$

$$0 = \frac{\mathrm{d}}{\mathrm{d}x}\left(\frac{\mathrm{d}P}{\mathrm{d}x} + \frac{1}{k_B T}P\frac{\mathrm{d}U_{tot}}{\mathrm{d}x}\right) \quad (68)$$

$$v = \left(\frac{f\,L}{k_B T}\right)^2\frac{D}{L}\left(e^{fL/k_B T} - 1 - \frac{f\,L}{k_B T}\right)^{-1} \quad (69)$$