1) Binding of substrates to proteins is a key process that underlies e.g. transport processes or enzymatic reactions.

a) Describe this process from a kinetic viewpoint and derive an expression for the bound-substrate fraction as a function of (free/unbound) substrate concentration.

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES$$

$$\frac{\partial ES}{\partial t} = k_1 E \cdot S - k_{-1} ES = 0$$

$$\frac{k_{-1}}{k_1} = \frac{E \cdot S}{ES}$$

bound fraction $\frac{ES}{ES + E} = \frac{k_1 S}{k_1 S + k_{-1}}$

b) If a chemical reaction happens after substrate binding we speak of Michaelis-Menten type kinetics. Derive an expression for the reaction speed as a function of the total protein- and (free/unbound) substrate concentration and assuming a steady-state concentration of the substrate bound state.

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

$$\begin{cases} \frac{\partial ES}{\partial t} = k_1 E \cdot S - (k_2 + k_{-1}) ES = 0 \\ E_T = E + ES \end{cases}$$

$$\Rightarrow k_1 S (E_T - ES) = (k_2 + k_{-1}) ES$$

$$ES = E_T \frac{S}{S + \frac{k_{-1} + k_2}{k_1}}$$

$$v = k_2 ES = k_2 E_T \frac{S}{S + \frac{k_{-1} + k_2}{k_1}}$$

2) Estimate the total work that the portal motor of ϕ29 needs to perform in order to overcome the entropy loss of the DNA after it has been packaged. (Hint: To count states, assume that the conformations of the DNA out of the capsid can be modeled by a freely jointed chain with 90° angles between the segments.). The length of the DNA is 6800 nm and the Kuhn length for bare DNA is roughly 300 bp.

* Entropy of DNA is capsid : $(\Omega = 1)$, $S = 0$

$300\text{ bp} \simeq 100\text{ nm}$

* Entropy of free DNA $(\Omega = 6^{\frac{6800}{100}})$

$$S = k_B 68 \ln 6$$

(Entropic) Work of packing: $-T\Delta S = k_B T\, 68 \ln 6$

3) The hydrolysis of maltose into glucose occurs slowly in the absence of a catalyst. The bacteria *E. coli* speeds up the reaction by three orders of magnitude by the enzymatic activity of specific proteins. Assuming Arrhenius behavior for the hydrolysis of maltose, to what fraction is the activation energy lowered by the protein machinery of *E.coli*?

absence of catalyst

$$k = A e^{-E/kT}$$

in *E. coli*

$$10^3 k = A e^{-E'/kT}$$

$$\Rightarrow 10^3 = e^{-(E'-E)/kT}$$

$$kT\, 3 \ln 10 = E - E'$$

fraction lowered $\dfrac{E-E'}{E} = \dfrac{kT\, 3\ln 10}{E}$

4a) The persistence length of a polymer is in essence the competition between entropic parts of the free energy and the energetic cost of bending a polymer. Explain this.

*1) Bending of a polymer cost energy and decreases with distance along the polymer*
*2) Bending of a polymer increases the energy and increases with distance along the polymer*
*The major polymer configuration is minimal in free energy (G=E-TS) and leads to competition between energy and entropy. At distances below the persistence length the polymer is unbended and above the persistence length it is bended.*

4b) A flexible polymer can be stretched and subsequently relaxed. Describe the relaxation process in relation to the concept of "Entropic Force".

*A relaxed polymer is in a state of higher entropy than a stretched one (fully stretched has only a single state). As a consequence, when the polymer is stretched it has a higher probability to relax back to the relaxed polymer configuration as oppose to stay in the stretched state. The driving force is entropy, hence the "Entropic Force"*

5a) Under certain conditions RNA polymerase hydrolyzes ATP at a rate of 10 molecules/s. Calculate the velocity of the polymerase, assuming that the distance between base pairs is 0.34 nm, that the polymerase steps from one base to the next and that in each step one ATP molecule is hydrolyzed.

$10 \text{ ATP/s} \cdot 0{,}34 \text{ nm/ATP} = 3{,}4 \text{ nm/s}$

5b) Estimate the amount of work performed in 1 minute when a kinesin molecule moves a vesicle over a microtubule. Use Stokes' formula (force = v 6πηr ) and the following information: vesicle radius = 1 µm, vinesin velocity = 450 nm/s, viscosity of the solution η = 0.01 g $cm^{-1} s^{-1}$, hydrolysis rate of kinesin = 80 ATP/s. The free energy of ATP hydrolysis is 30 kJ/mol. Also discuss whether there is enough free energy to do this work.

Drag Force $= 450 \cdot 10^{-7} \text{ cm/s} \; 6\pi \; 10^{-5} \text{ kg cm}^{-1} \text{s}^{-1} \cdot 10^{-6} \text{ m}$

$= 8{,}5 \cdot 10^{-15} \text{ N}$

distance in 1 min: $450 \cdot 10^{-9} \cdot 60 = 27 \cdot 10^{-6} \text{ m}$

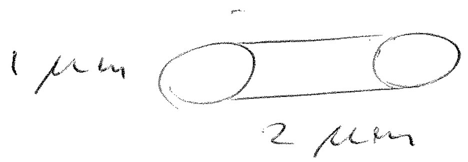
Work $= F \cdot d = 8{,}5 \cdot 10^{-15} \text{ N} \cdot 27 \cdot 10^{-6} = 23 \; 10^{-20} \text{ J}$

Energy of ATP hydrolysis

$$\frac{60 \cdot 80 \cdot 30 \cdot 10^{3}}{6 \cdot 10^{23}} = 24 \cdot 10^{-17} \text{ J}$$

⇒ There is enough energy by the hydrolysis of ATP

6) Estimate the total number of membrane proteins in *E. coli*




Area E. Coli : $2 \cdot 1 + 2\pi\left(\frac{1}{2}\right)^2 \simeq 4\ \mu m^2$

Area single protein : $2\ nm \cdot 2\ nm = 4\ nm^2$

~ 50 % of cell area is protein

$$\frac{\frac{1}{2} \cdot 4 \cdot \mu m^2}{4 \cdot 10^{-6}\ \mu m^2} \simeq 10^5 - 10^6 \text{ proteins}$$

7) The insertion of small peptides into the plasma membrane occurs spontaneously. What can be said about the entropic driving force for insertion?

$\Delta G < 0$

$\Delta H - T\Delta S < 0$

$\Rightarrow \Delta S > \frac{\Delta H}{T}$