Final exam

First name: _____ **Last name:** _____

Student number ("Matrikelnummer"): _____

- Please write your name on the exam and keep an ID card ready.
- You may use a **calculator** (but no computer or smart phone), a **dictionary**, and one page (**"Spickzettel"**, front and back) with your notes.
- The exam is closed book, i.e. you are not allowed to consult books or other references.
- You have 120 min for the exam.
- Please only write on the handed out exam sheet. You may use both sides of the pages.
- Good luck!

Problem	Your points	Maximal points
1		10
2		14
3		11
4		12
5		8
6		10
7		8
8		10
9		10
10		7
Σ		100

Some useful constants
Atomic mass unit: $u = 1.66 \cdot 10^{-27}$ kg
Elementary charge unit: $e = 1.6 \cdot 10^{-19}$ C
Gas constant: $R = 1.9872036 \cdot 10^{-3}$ kcal/(K·mol)

Name: _____

Problem 1

Anfinsen's Hypothesis.

a) State Christian Anfinsen's hypothesis or "dogma" for (globular) proteins.

b) Briefly describe two observations or molecular mechanisms that violate or contradict Anfinsen's hypothesis.

Name: _____

Problem 2

Length scales. Give the (approximate) length scales of the following structures or objects:

a) Distance from the hydrogen-donor to the hydrogen-acceptor in a hydrogen bond.

b) Diameter of a standard B-form DNA helix.

c) Diameter of a small globular protein.

d) Cross-sectional diameter of a microtubule.

e) Size of a prokaryotic cell.

f) Which of the length scales a)-e) can be resolved with super-resolution fluorescence microscopy?

g) Which of the length scales a)-e) can be resolved by X-rays?

Name: _____

Problem 3

Single and double-stranded λ-phage DNA. The λ-phage (which is essentially a virus that preys on *E. coli*) has a 48.5 kbp double-stranded DNA genome. Purified λ-phage DNA ("λ-DNA") is frequently used in biophysical experiments. Let us consider some properties of both single- and double-stranded λ-DNA in aqueous solution at (roughly) physiological salt concentration ($\approx$ 150 mM monovalent salt) and pH. By single-stranded λ-DNA we mean that only one strand (of 48500 bases) is present. You can consider both single- and double-stranded λ-DNA to be well approximated by the FJC model in this problem.

a) What is the contour length of double-stranded λ-DNA?

b) What is the contour length of single-stranded λ-DNA? You can assume that the length per base for single-stranded DNA is 0.5 nm. Is the contour length for single-stranded λ-DNA shorter or longer than the length in a)? Why?

Name: _____

c) What is the average root-mean-squared end-to-end distance of double-stranded λ-DNA in free solution?

d) What is the average root-mean-squared end-to-end distance of single-stranded λ-DNA in free solution? You can assume that the segment or Kuhn length for single-stranded DNA is 1 nm.

e) Sketch the force-extension behaviour of both single- and double-stranded λ-DNA in a coordinate system with the force on the y-axis and extension on the x-axis. Clearly label which curve is for single-stranded and which curve is for double-stranded DNA.

Name: _____

Problem 4

Optimizing Michaelis-Menten. You are part of a team that is working on improving an industrial process for bioethanol production that used an enzyme E that is used in to break down its substrate (S) cellulose into the product (P) glucose. The overall reaction is well approximated by Michaelis-Mention kinetics and described by the following reaction scheme:

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

The process is currently run with a substrate concentration of 10 mM and an enzyme concentration of 1 μM. The standard enzyme currently used in the process has been characterized and the following parameters have been determined:

Parameter	Value
k_1	10^5 s^{-1} M^{-1}
k_{-1}	10^{-5} s^{-1}
k_2	10 s^{-1}

Your team members suggest several strategies to increase the rate of product formation in the reaction process. Which of these strategies would you pursue as promising candidates to significantly enhance the rate of product formation **and why**? With each change discussed below, we assume that the other parameters stay at their current values.

a) Make a mutation to the enzyme that increases k_1 10-fold.

Name: _____

b) Make a mutation to the enzyme that increases k_2 10-fold.

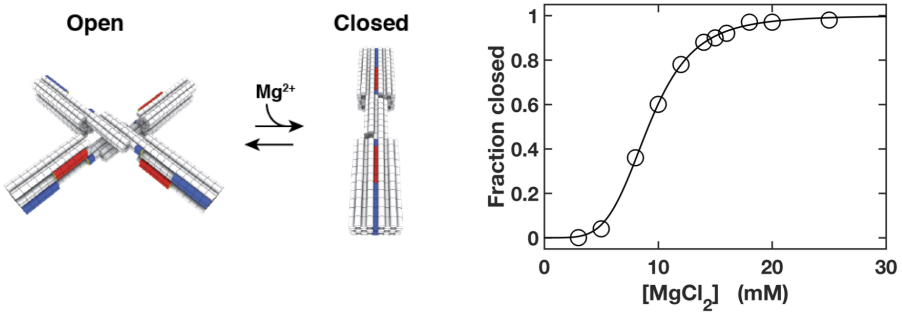
c) Increase the substrate concentration 10-fold.

d) Increase the enzyme concentration 10-fold.

Name: _____

Problem 5

DNA electrostatics. A DNA origami structure consist of two large arms that are connected by a flexibel pivot or junction (see schematic on the left below). This DNA switch can be in an X-shaped open state or in a more compact closed conformation. The closed conformation is stabilized by short range DNA stacking interactions of the elements that are shown in red and blue in the schematic below. Since the switch device is constructed from DNA, there is considerable electrostatic repulsion between the two arms; consequently, in low salt (< 5 mM $MgCl_2$) the open state is predominantly populated and in high salt (> 15 mM $MgCl_2$) the switch is mostly in the closed state. Using SAXS data, we have determined the relative fraction of DNA switches in the closed state as a function of $MgCl_2$ concentration (black symbols in the plot on the right).



a) You want to fit the data for the relative fraction of DNA switches in the closed state as a function of $MgCl_2$ concentration with a simple analytical model (similar to the solid line in the panel on the right). What model could you use? Describe the free fitting parameters of the model and their interpretation. Hint: You could consider Mg^{2+} as a ligand that binds to the origami structure.

Name: _____

b) A colleague wants to repeat a similar measurement with NaCl instead of $MgCl_2$. He expects the midpoint of the transition (i.e. the salt concentration where the fraction closed is 0.5) to be $\approx$ 20 mM NaCl (instead of $\approx$ 10 mM for $MgCl_2$) since Na^+ ions have only half the charge of the Mg^{2+} ions. Is this a realistic expectation? Why or why not?

c) Why would you expect Poisson-Boltzmann theory to give more accurate results for mono- as compared to divalent ions for calculations of nucleic acid electrostatics?

Name: _____

Problem 6

Reaction kinetics. Let's have a look at a reaction that consists of two consecutive, irreversible elementary steps:

$$A \xrightarrow{k_1} B \xrightarrow{k_2} C \tag{2}$$

Assume now that such process is a subsequent radioactive decay. We can see immediately that the following initial conditions at $t = 0$ hold: $[A] = [A]_0$, $[B] = 0$, and $[C] = 0$.

a) Write down the conservation equation valid at all times that links $[A]$, $[B]$, and $[C]$.

b) Using the information from above, set up the rate equations for the process and solve them to determine $[A]$, $[B]$, and $[C]$ as a function of time.

Name: _____

c) Now consider two special cases. Case 1: $k_1 >> k_2$ and Case 2: $k_2 >> k_1$. Briefly explain what happens in these cases and write down the simplified equation for $[C]$, respectively. Also state which step becomes the rate-limiting one in each case.

d) For the case where $k_2 >> k_1$, sketch concentration vs. time for A, B, and C.

Name: _____

Problem 7

Nucleic Acids.

a) What are typical values for: Strength of a hydrogen bond, linear charge density for a DNA duplex (!!), and binding strength of a base pair.

b) What are typical values of k_{on} for a diffusion-limited reaction in aqueous solution? What are typical values of k_{on} for DNA hybridization reactions? Is DNA hybridization a diffusion-limited or reaction-limited reaction?

Name: _____

c) Briefly describe the process of DNA hybridization and dissociation between two short DNA molecules (also called oligonucleotides). What is different for longer DNA molecules?

d) Describe at least two different methods for experimentally determining the association and dissociation behavior of DNA molecules.

Name: _____

Problem 8

DNA thermodynamics and kinetics. Use the following values to perform calculations in this problem.

Sequenz	ΔH^0 (kcal/mol)	ΔS^0 (cal/mol·K)	ΔG^0_{37} (kcal/mol)
AA/TT	-7,6	-21,3	-1,00
AT/TA	-7,2	-20,4	-0,88
TA/AT	-7,2	-21,3	-0,58
CA/GT	-8,5	-22,7	-1,45
GT/CA	-8,4	-22,4	-1,44
CT/GA	-7,8	-21,0	-1,28
GA/CT	-8,2	-22,2	-1,30
CG/GC	-10,6	-27,2	-2,17
GC/CG	-9,8	-24,4	-2,24
GG/CC	-8,0	-19,9	-1,84
Initiation	+0,2	-5,7	+1,96
AT-Terminus Korrektur	+2,2	+6,9	+0,05
Symmetrie-Korrektur	0,0	-1,4	+0,43

a) Calculate the free energy (total ΔG^0_{37}) for the following DNA duplex using the nearest neighbor model using standard conditions (1 M NaCl, 37 °C):

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5'-GTAATGAAGA-3'
3'-CATTACTTCT-5'
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Name: _____

b) Based on the obtained free energy value for the duplex from a), estimate the off-rate k_{off} at 37 °C, assuming an on-rate of $k_{on} = 10^6(\text{Ms})^{-1}$.

c) Roughly sketch the melting profile (Amount of unpaired base pairs vs. temperature) and mark the melting temperature (you don't need to calculate numeric values!). How is the melting temperature defined?

Name: _____

Problem 9

Filaments.

a) Describe the internal structure of actin filaments and of microtubuli.

b) What is their typical role inside a cell?

c) Using the case of actin polymerization: Describe the difference between a diffusion-limited and a reaction-limited reaction.

Name: _____

d) Describe the assembly and disassembly process of actin filaments.

e) What is known as dynamic instability in the context of microtubular assembly? Sketch length vs. time during polymerization and depolymerization of microtubuli and briefly explain what is know as *rescue* and *catastrophe*.

Name: _____

Problem 10

Scanning Probe Techniques.

a) Briefly explain how an Atomic Force Microscope (AFM) works.

b) Let's assume the interaction potential between an AFM cantilever and the surface of a sample is $V(d) = 4\epsilon\left((\sigma/d)^{12} - (\sigma/d)^{6}\right)$ with d being the distance between the tip and the sample.

Name and sketch the potential, mark σ and ϵ and briefly describe the different regimes.

Name: _____

c) Sketch a force vs. distance curve obtained when performing a force spectroscopy experiment with a protein using an AFM and explain the different features observed.