Final Exam

KBB032

Biochemistry and Molecular Biology

Time and location: Monday, January 3rd, 2022, morning session 08.30-12.30

Teacher and examiner: Michaela Wenzel 772 2074

Teacher will be available for questions by phone throughout the exam.

Aids:	All aids are allowed, including laptops and lecture notes. See rules on next page.
Exam review:	Results will be reported to Ladok within three weeks. Grading of the exam may be reviewed after agreement with Michaela Wenzel.
Points breakdown:	Biochemistry questions: 50 points
	Molecular Biology: 50 points
	Points for each question are indicated in parentheses.
	Grading: 50% = 3; 65% = 4; 80% = 5

Read all questions carefully!

Remember that some questions may have more than one correct answer.

Please write legibly.

Instructions for using laptops:

- A computer is allowed.
- You must be certain to turn your sound off
- You must shut down all modes of communication with other students (eg. chats, push notices, slack, email, twitter etc). It is not allowed to communicate with another **person** by any means throughout the exam. The only exception is a supervised phone call to the examiner.
- Everything we ask will be possible to answer based on the course contents that are published in Canvas.
- You will not need to use any other source of information. However, it is not prohibited to search the internet.
- If you use web sources for any kind of information, be careful to avoid plagiarizing. Write a reference to the source (so we can look it up). Use your own words to describe the information in the source to show you have understood it; do not copy text from the source.

Question 1: Definitions and concepts (10 p total)

Below is a short explanation of different expressions and phenomena used in biology. Name the concept that is described (1 p each).

- a) the theory that mitochondria and chloroplasts originate from prokaryotes that has been incorporated in eukaryotes
- b) a chemical reaction characterized by the gaining of electrons by an atom
- c) a chemical reaction characterized by the loss of electrons from an atom
- d) a group of genes that is regulated by the same regulator but not necessarily located close to each other on the chromosome
- e) an end-product in a pathway will inhibit an enzyme in the beginning of the same pathway
- f) amino acids that a given organism needs but cannot make itself and therefore must be obtained from the diet
- g) Transformation of (to most organisms) inaccessible N₂ to accessible NH₃ by certain specialized bacteria
- h) a catalytic step in a metabolic pathway that determines the reaction speed of the whole pathway
- i) the transfer of a phosphate group from a substrate to ADP
- j) the transfer of a phosphate group to ADP that is driven by the transmembrane proton gradient

Question 2: Chloroplasts and mitochondria (9 p total)

- a) Name the key membrane-bound process that take place in mitochondria and chloroplasts, respectively (1 p each).
- b) Which pathway that occurs in the respective organelle's lumen is closely coupled to and dependent on these membrane reactions (1 p each)?
- c) What is the predominant role of each organelle in cellular metabolism (1 p each)?
- d) Why do plants have both of these organelles and how are their functions connected (1 p each)?
- e) In plants, mitochondria use organic molecules produced by chloroplasts. Where do non-photosynthetic organisms obtain this compound from (1 p)?

Question 3: Pentose phosphate pathway (6 p total)

- a) Which are the two most important functions of the pentose phosphate pathway? (4 p)
- **b**) Glucose-6 phosphate dehydrogenase is a key enzyme of this pathway, describe the reaction catalysed by this enzyme. (2 p)

Question 4: Oxidative phosphorylation (7 p total)

In aerobic organisms cellular respiration is driven by oxidative phosphorylation, which generates ATP.

- a) Where does this process take place in eukaryotes and prokaryotes, respectively (1 p each)?
- b) Which force is used by the ATP synthase complex to form ATP and how is this force formed/maintained (1 p)?
- c) The electron transport chain can be disrupted by two classes of molecules, inhibitors and uncouplers. What is the difference between these two? Which one is more likely to result in the formation of reactive oxygen species and why (4 p)?

Question 5: Photosynthesis (9 p total)

Photosynthesis and its light reactions in green plants and certain microbes is a very important process for life on earth.

- a) Different organisms have evolved a range of different photosynthetic pigments. How do they differ and what is the reason behind this diversity (2 p)?
- b) Which steps in photosynthesis require light and why (what is the role of the light that makes the reaction possible) (4 p)?
- c) What are the end products of photosynthesis and for which other processes or pathways are they used (3 p)?

Question 6: DNA and RNA: Differences between prokaryotes and eukaryotes (6 p total)

- a) Describe the major difference between a prokaryotic and eukaryotic gene and the consequences for the structure of the resulting mRNA.
- **b**) Describe the three key processing processes in eukaryotes and briefly describe why they are necessary.

Question 7: Translation (10 p total)

Imagine that you have inhibitors of bacterial translation that interfere with different steps with the pathway. For each of the situations below, what would you observe, if you would characterize the proteome of the cells and what would happen to the ribosome (1 p each)?

Think about these carefully!

- a) The inhibitor binds to the 16S rRNA that recognizes the RBS sequence in the mRNA.
- b) The inhibitor binds to the exit site of the ribosome.
- c) The inhibitor interferes with the synthesis of fMet-tRNA.
- d) The inhibitor prevents binding of RF1 or 2.
- e) The inhibitor prevents binding of RF3.
- f) The inhibitor prevents binding of IF1 and 2.
- g) The inhibitor binds to and inhibits EF-Ts.
- h) The inhibitor prevents formation of the peptide bond.
- i) The inhibitor prevents the movement of the ribosome along the mRNA.
- j) The inhibitor allows binding of incorrect tRNAs to the codons on the mRNA.

Question 8: PCR and sequencing (10 p total)

Polymerase chain reaction and DNA sequencing are based on the same molecular principles. However, there are crucial differences that make the respective reactions possible.

- a) Describe the principle of the polymerase chain reaction. Which components are needed for the reaction to be successful and what is the role of each component (2 p)? What are the individual phases and what happens in each phase (2 p)?
- b) What could be a reason for a PCR not yielding any product other than forgetting to add one of the components (2 p)?
- c) Describe the principle of Sanger sequencing including components and point out the four major differences to a PCR reaction (2 p).
- d) Explain the role of each of these differences that enables sequencing (2 p).

Question 9: Sequences (9 p total)

Translate the given sequence into the missing corresponding sequences (genetic code for reference can be found here: <u>https://jgi.doe.gov/wp-content/uploads/2016/03/1035px-Aminoacids_table.png</u>).

Note that depending on which sequence is given there could be more than one correct answer. Mind the orientation!

(1 p per translated sequence)

DNA sequence (5'-3'): ATGCGAATTGCAATAAGCGGAGGCGGTACA

Complementary strand (5'-3'):

RNA sequence (5'-3'):

Protein sequence (N-C):

DNA sequence (5'-3'):

Complementary strand (5'-3'):

RNA sequence (5'-3'): AUGACUGAAC AAACCAUUGC ACAUAAACAA

Protein sequence (N-C):

DNA sequence (5'-3'): Complementary strand (5'-3'): RNA sequence (5'-3'): Protein sequence (N-C): MLEQVILFTI LMGFLISVLL

Question 10: Measuring RNA levels (12 p total)

Gene expression can be measured on DNA, RNA, protein, and metabolite level. Global profiling on mRNA level has continued to be a popular approach, which has seen an interesting evolution of methodology.

- a) Describe the concepts of RNA microarrays, RNA sequencing, and ribosome sequencing (2 p each).
- b) What are the critical differences between these three methods (3 p)?
- c) What is the main advantage of RNA sequencing over microarrays (1 p)?
- d) When would you use RNA sequencing and when ribosome sequencing (2 p)?

Question 11: Measuring gene expression (12 p total)

With your knowledge about methods to measure gene expression, how would you approach the following research questions? Motivate your answer and explain your experimental setup. Keep in mind that there can be more than one feasible approach.

- a) You want to know at which time during embryonic development a specific (known) gene regulator is active. Your model organism is zebrafish. (2 p)
- b) You suspect that a genetic predisposition (you don't know which one) causes a higher chance for suffering from long Covid. How would you attempt to identify the involved genetic markers? Funding for Covid research is abundant and have no limitations regarding accessibility or cost of the chosen method. (5 p)
- c) A low-income country registers an outbreak of an infectious disease among the rural population. You are tasked to identify the causative agent with a simple and cheap approach that is feasible to implement in a mobile low-tech lab. Based on symptoms and epidemiology, you can narrow down the causative agent to a handful of possible culprits. Which method and instrumentation would you recommend toidentify the infectious agent in this setting? Once identified, which technique could you use for large-scale diagnostics? (5 p)