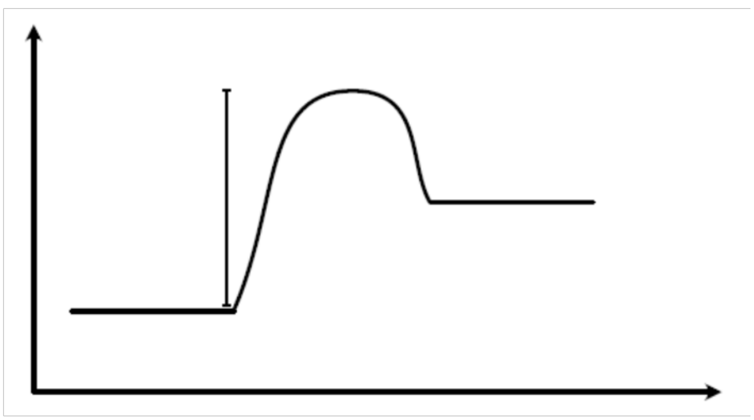
Exam:

1	Why is the citric acid cycle considered amphibolic? Provide examples of its anabolic and catabolic roles.	**3 P**

2	A) Below you can see a reaction diagram. Please label six important elements, including the axes, with the correct terms. B) What changes if this reaction is catalyzed (e.g. by an enzyme)? Please draw this in the diagram. C) Will this reaction be endo- or exothermic? Explain your conclusion.	**3 P**



3	Order the following peptides by their (i) isoelectric point (pI), (ii) molecular weight (MW) and (iii) extinction coefficient, all in increasing order: (A) MRWKGFYAKKHRVLQHK (B) MDEILFDEEAVYDS (C) MRSDAWVGKEAWTGIVAMYEK	**3 P**

4	(A) Draw glycine and proline amino acids at physiological pH 7.4. Include all atoms, valence electrons and charges of the most abundant form. (B) Give two reasons why replacing a Glycine with Proline in a protein might destabilize or disrupt secondary structure and fold.	**3 P**

5	(A) Draw the chemical structure of a peptide bond. You can mark the side chains of the amino acid as R or Rest. (B) Name one amino acid that falls into each of these categories (you cannot name the same amino acid twice) ● hydrophilic side chain ● hydrophobic side chain ● side chain contains a nitrogen atom ● side chain contains a sulphur atom	**3 P**

6	(A) Describe three interatomic interactions or effects that drive protein folding! (B) For each interaction, is it an enthalpic or entropic contribution to protein folding?	**3 P**

7	An AlphaFold prediction predicts a direct interaction between protein A and protein B. A) What experimental technique would you use to test whether this is indeed the case? Explain briefly how this technique works. B) You are successful in confirming the predicted interaction experimentally. How would you confirm that the interaction occurs at the protein-protein interface predicted by AlphaFold?	**3 P**

8	What are the three main filamentous structures of the eukaryotic cytoskeleton. Give a major function for each one.	**3 P**

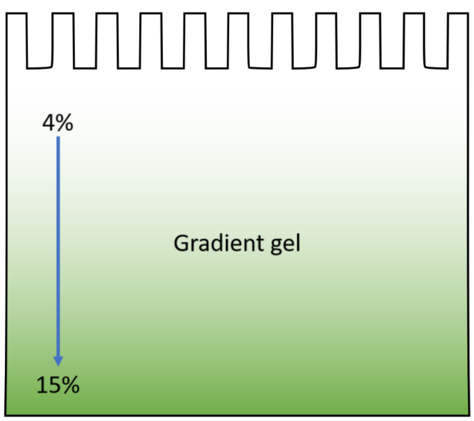
9	You are studying the import of the model protein DHFR into mitochondria and the nucleus. The model protein carries a mitochondrial targeting signal at its N-terminus and a nuclear localization signal at its C-terminus. In the presence of methotrexate, which stabilizes the folded state of DHFR, into which organelle(s) is the protein imported? Explain your answer, giving the reasons for import or lack of import into the respective organelles.	**3 P**

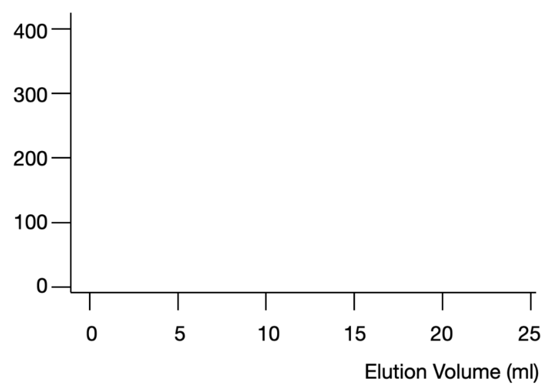
10	In the cell, the folding of many proteins is assisted by helper enzymes. Please name the corresponding enzyme class and describe its function briefly.	**2 P**

11	Name the four phases of the cell cycle and briefly describe the main function of each phase.	**4 P**

12	You generated a knock-out cell line of a serine protease using the CRISPR/Cas9 technology. You observe that the knock-out cell line is extremely sensitive to UV light exposure. A) How would you confirm that the UV sensitivity is caused by the knock-out you generated and not by a an off-target effect/secondary mutation? B) How would you test whether the catalytic activity of the serine protease is required to provide resistance to UV-light?	**3 P**

13	As part of a new research project, you are asked to study a protein complex composed of three subunits with molecular weights of 100 kDa, 60 kDa, and 25 kDa. You have sufficient amounts of purified protein and decide to begin with basic biochemical characterization. You plan to analyze the sample using SDS-PAGE and size-exclusion chromatography (SEC). A: In the schematic on the left above, indicate how the three proteins would be expected to resolve on an SDS-PAGE gel. Clearly mark the *relative* positions of each protein band. B: In the schematic on the right hand side, illustrate the expected SEC chromatogram for this sample. Indicate the relative elution positions of the individual proteins and the trimeric protein complex. C: What additional reagent or method is required to visualize ("see") the proteins on the SDS-PAGE gel? D: How would you label the y-axis of the SEC chromatogram? In other words, what measurement is used to detect protein abundance in size-exclusion chromatography?	**4 P**





14	You need a specific purified protein in order to run a biochemical assay. Unfortunately, the protein is only expressed in baby Panda bears, therefore, purifying the protein from its natural source (baby Panda bear!) is not an option and you decide to clone the coding sequence into a vector to enable the recombinant expression of the Panda protein for your assays: (A) You receive baby Panda cDNA and genomic DNA which you can use to amplify the DNA sequence coding for your protein. What method are you using to obtain enough DNA for the following cloning steps and which DNA prep (cDNA or genomic DNA) are you going to use (justify your choice)? (B) You know that your Panda protein is globular and not a membrane protein. However, it contains a cleavable signal sequence (ER targeting), numerous cysteines and potential glycosylation sides. After signal sequence cleavage your protein should have a molecular weight of 25 kDa. Which expression system (*E. coli*, *S. cerevisiae* or *H. sapiens* cells) would you choose and why?	**3 P**

15	You have successfully cloned the DNA sequence encoding the baby Panda protein into an expression plasmid (see question 14). Now you want to engineer the plasmid further to help with the purification of the protein. (A) Which affinity tag would you engineer into your recombinant protein? Would you use N-terminal or C-terminal tagging (justify your choice)? (B) In addition to the affinity tag, you would like to have a specific protease cleavage site between the affinity tag and your protein, so that you can produce native-like Panda protein. How can you engineer such a protease site into your construct? (C) To check your prep from human cells, you run a denaturing SDS-PAGE of your Panda protein preparation under reducing and non-reducing conditions. You expect a molecular weight of 25 kDa for the protein following cleavage of the signal sequence. However, the gel shows under reducing conditions a 30 kDa band and under non-reducing conditions a 120 kDa band. What do you conclude?	**3 P**

16	Briefly describe the central aspect of Darwin's theory of the evolution of species.	**2 P**

17	You have a pair of proteins that are orthologs. What does that mean?	**2 P**

18	Evolution favored a genetic code of triplet codons for 20 canonical amino acids. A: How many different codons can be formed using triplets and the four different bases? How many of those codons code for amino acids? B: How many codons are recognized by tRNAs? How many are recognized by proteins? C: Why don't we use a doublet code?	**3 P**

19	The Z-score is a data transformation often used to display values in heatmaps. It is calculated by subtracting the population mean (μ) from an observed value (x) and dividing the result by the population's standard deviation (σ). What does a Z-score of 3 mean? $Z = \frac{x - \mu}{\sigma}$	**2 P**

20	In an experiment you measure three continuous properties (p1, p2, p3) of a mysterious substance X at 10 timepoints. In your data analysis you find that p1 is positively correlated to p2 ($r > 0.8$) but negatively correlated to p3 ($r < -0.8$). What can you say about the correlation between p2 and p3?	**2 P**

21	A reaction described by the time-evolution f(t) = 1-exp(-t/tau) has a substrate turn-over rate of k=1/tau=1/(60 minutes). (i) How much substrate is turned over after 120 minutes? (ii) How long does it take until 95% of the substrate is turned over?	**3 P**