Name: **Student number:**

CHEM-E8115 Cell Factory, Examination April 13th, 2021

1. Open book exam, duration: 4 hours
2. Your answers should be based on the subjects discussed in the course!
3. In order to pass the exam you need 40% of the total points (20 out of 50 points). The exam contributes 70% to the final grade. The extra points from the weekly assignments count towards the exam.
4. Return your answers (marked with name and student number) as a single PDF file via MyCourses. If problems during submission, email your answers to alexander.frey@aalto.fi

Question 1 (max. 15 points)

You are responsible for the establishment of a new production facility for N-glycosylated proteins in a biotechnology company. The company produces proteins for a wide range of applications ranging from industrial bulk enzymes to therapeutic proteins. You have to inform the company management about different cellular systems available and convince them, in which of these they should invest.

Describe the pros and cons of two different cellular systems, which are suitable for production of N-glycosylated proteins and give the reasons why you would choose a particular system. Your discussion should include a wide range of criteria that can affect the choice. In total, the length of this essay should be between 1 to 1.5 pages of text.

Question 2 (max. 15 points)

Besides Ethanol yeast is producing naturally a number of higher alcohols, such as isobutanol, isoamylalcohol or 3-methyl-1-butanol. Typically, these alcohols are produced at very low concentrations, but using genetic engineering the amounts produced have increased. Two key enzymes in these engineered strains are *Lactococcus lactis* 2-keto-acid decarboxylase (KIVD) and the NADPH-dependent medium chain alcohol dehydrogenase ADH6 from *S. cerevisiae*. Both enzymes have a broad substrate specificity.

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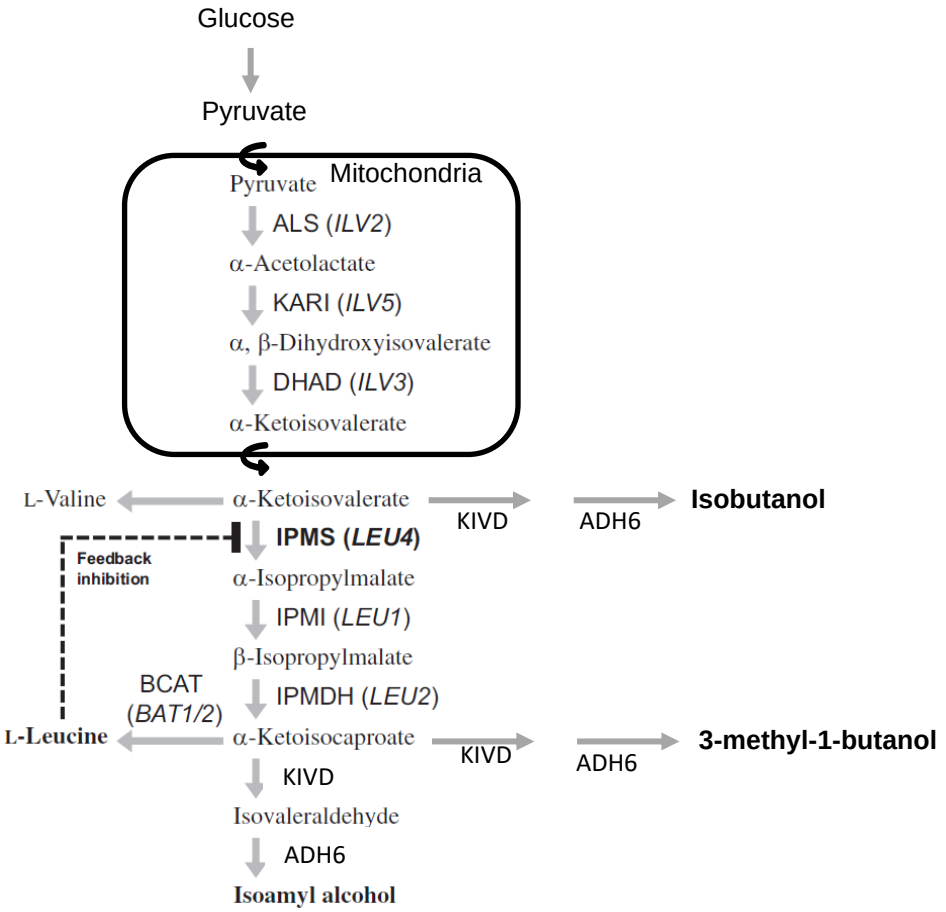

Discuss and explain the following three statements, the questions should direct your answers. In total, the answers should be between 1 to 1.5 pages of text:

a) "If ILV2, ILV5 and ILV3 activities were retargeted to the cytoplasm, the yields of isobutanol would increase." What would be the potential advantages / disadvantages? How could this retargeting be achieved? (max. 4 points)
b) "Replacing ADH6 with an NADH-dependent alcohol dehydrogenase would increase productivity" Is it a good idea? Why / why not? How could it be achieved? Are there alternatives to deal with the cofactor specificity of an enzyme? (max. 4 points)
c) "Feeding of α-ketoisovalerate to the growing cultures increases the yield of isobutanol, but not of the two other products." Why? How could this problem be solved? (max. 4 points)
d) What other genetic modifications could be introduced to further increase production of these alcohols? List and discuss. (max. 3 points)

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Question 3 (max. 10 points)

The pET expression system is derived from the *E. coli* bacteriophage T7.

a. Describe all the elements of the pET expression system and how it is used in *E. coli* to express recombinant proteins (2 points).
b. Describe in detail how the pET expression system must be modified to express proteins in another prokaryote and a eukaryote (8 points).

Question 4 Shortly describe/explain (2 points per question, in total 10 points)

a) explain the similarities and difference(s) between a replicative and integrative plasmid
b) What are the advantages of using inducible promoters compared to constitutive promoters?
c) Which genetic elements can be utilized in order to keep the chromatin in an open conformation? How do they function?
d) explain the rationale behind "laboratory evolution approaches" for strain engineering.
e) explain the rationale behind activating the unfolded protein response for enhancing recombinant protein production.