Name: **Student number:**

CHEM-E8115 Cell Factory, Examination February 26th, 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)

Your company is investigating the possible use of genetically-engineered cells to produce a vaccine against SARS-CoV 2 virus. As vaccines, two different fragments of the viral spike protein (S protein) which is located on the surface of the virus are considered. Fragment 1 contains the N-terminal part of the S protein. Fragment 2 encompasses the central part of the S protein. Fragment 1 contains several N-glycosylation sites and one sequential disulfide bond. Fragment 2 is non-glycosylated but contains several non-sequential disulfide bonds. Both fragments are soluble, ie. not embedded into the membrane. To enable rapid production the proteins should be secreted.

a. List and discuss the pros and cons of using *prokaryotes (gram- and gram+), Saccharomyces*, and mammalian cell lines to produce the two protein fragments that could be used as vaccines. (max. 10 points)
b. Pick the organism you deem most suitable for production of the two fragments and explain how cell engineering could help to increase productivity. In case you suggest two different production systems for the two proteins, describe the needed engineering steps for both. (max. 5 points)

Question 2 (max. 15 points)

Production of biodiesel in yeast. The figure depicts a sketch of the simplified metabolic pathway leading to formation of palmitate ethyl ester, which can be utilized as biodiesel. The steps take place in the cytoplasm and mitochondria.

The overall reaction balance from acetyl-CoA to palmitate is: 8 Acetyl-CoA + 7 ATP +14 NADPH -> palmitate + 14 $NADP^+$ + 8 CoA + 6 H_20 + 7 ADP +7 P_i. Formation of palmitate ethyl ester requires equimolar amounts of ethanol and palmitate.

The conversion of acetate into acetyl-CoA is carried out by acetyl-CoA synthetase (ACS). ACS is inhibited by acetylation. The endogenous wax ester synthase (WS) has very low activity.

Feeding experiments have been performed according to the matrix in which acetate, ethanol and palmitate were added to the cultivation medium and their effects on palmitate ethyl ester production were determined.

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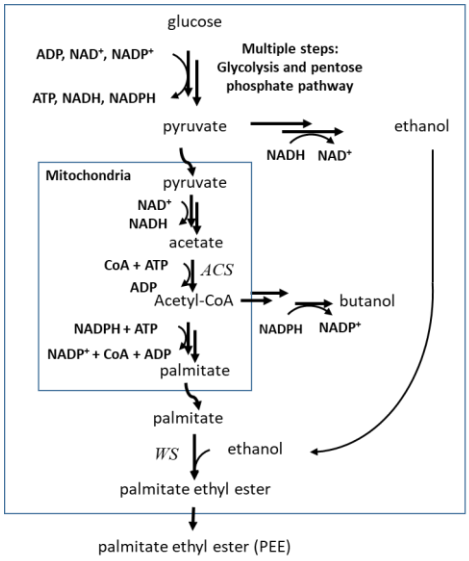

Feeding matrix

	ethanol	acetate	palmitate
ethanol	0	+	++
acetate	+	+	++
palmitate	++	++	++

0: no effect; +: positive effect; ++: strong positive effect

Single compounds or a combination of two compounds according to the matrix were supplemented to the media. Ethanol plus Ethanol combination indicates that only ethanol was added, Ethanol plus acetate combination indicates that ethanol and acetate was added, etc.

a) Which are the possible rate-limiting steps in this metabolic pathway? Explain your reasoning. (max. 6 points)
b) Propose a suitable genetic engineering strategy to overcome the encountered bottlenecks. (max. 9 points)

Question 3 (max. 10 points)

The pL expression system is derived from *E. coli* bacteriophage λ, a phage which cannot infect other bacteria than E. coli.

a. Describe all the elements of the pL expression system and how it is used for protein expression (2 point).
b. Describe a strategy and the necessary modifications to the pL expression system that allow its use in another prokaryote and a eukaryote. Give full details of these modifications (8 points).

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

a) Why isn't the plasmid that contains the strongest promoter always the best option for expression of a protein?
b) describe the basics of *Agrobacterium tumefaciens* mediated plant transformation
c) What are the main differences between a molecular chaperone and a protein disulfide isomerase?
d) describe the difference between feedback inhibition and repression
e) What is the motivation for increasing genetic diversity when engineering cell factories?