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Evaluation of Bachelor Thesis

author of BT: Eliška Kotounová

Supervisor: Zdeněk Franta, PhD
Školitel-specialista: Ivana Kutá-Smatanová, PhD

Název BP: Mechanism of dsRNA virus relpication: cloning, production and strutural characterisation of N-terminal domain of σNS

The bachelor thesis of Eliška Kotounová comprises 53 pages, 5 introductory figures, 26 experimantal Figures, several tables, and appendices. The thesis represents today´s craft of basic protein biochemistry, with cloning, pilot expression experiments with subsequent scale-up, and pilot crystallisation. Eliška performed countless culturing, electrophoretic and immunodetection methods, most of which were performed and presented in an impecable way. Despite the thesis not having a happy ending, it is apparent that Eliška has mastered numerous laboratory techniques and interpreted scientific literature in an advacned way, so I am convinced that Eliška has all the requirments to become good and rigorous scientist. Even though I have not seen many, this one of the best bachelor theses I have read, mainly in its depth of **Introductory** section, detailed **Material and Methods**, clear **Results**, and down-to-Earth **Discussion**. Also, the written English presented in this thesis is of a very good standard.

I have only a few **comments (C)** that may help Eliška to prepare, next time, better thesis or any other written work. From technical point of view, I have only two **questions (Q)**.

Q1: You state (p33) that your protein gets proteolytically cleaved based on unexpected phoretic mobility result (lower MW), yet you used protease inhibitors during protein solubilisation and most of BL21 strains are genetic knock-outs of endogenous proteases *lon* and *ompT*. How do you explain that or what kind of *E. coli* protease would be responsible for such a cleavage, e.g. is there any sequential homologue of yeast SUMO protease (ULP1) in *E. coli*? Would you consider denaturing conditions (e.g. 8M urea) beneficial to purify your protein (and potentially having intact protease) and then refold your protein to native state, which should work well due to high solubility of the SUMO tag?

Q2: Why do you use two different buffer compositions for solubilisation of pilot experiemnts (p16) and for protein purification (p18)? I am expecially puzzled why you included 10mM imidazole for pilot expression and omitted for purification, I would tend to see an opposit logic in design of buffer compositions. The pH shift was made due to pI of your protein fragment?

C1/Q: I noticed you monitored your bacterial culture at 550 nm as opposed to standard 600 nm, is there any reason/advantage for that?

C2: Please indicate centrifugal speed in *g*´s and not rpm´s (through out the whole thesis).

C3: Unify your bold pattern in all Figure legends (Figure 1–5 vs the rest) / Unify the symbol usage after Figure number - sometimes you use colon, the other time you use a dash (Figure 6 / Figure 7).

C4: Label Figures in multipanel Figure in the top right corner: A), B),...

C5: Always remember to use negative controls for colony PCRs.

C6: It is customary that Western Blots match SDS-PAGE figure, which is not the case for Figure 8 and 9, so this section may come across a bit hazy.

C7: It is customary to present DNA/RNA agarose gels on black backround.

C7: Remember that you always state what membrane you used for protein blotting: a) its type: nitrocellulose x PVDF and b) its pore size: 0.2 x 0.45 μm; especially, if you are onto small protein, you do not want to use bigger pores of the membrane to blot your protein through.

C8: Multiplication sign „times" has actually a symbol „×", please use next time instead of „x" or „X"

C9: The overuse of conjuctive adverbs like „however", „therefore",.. sometimes hinders the flow of readability. Please do not use „therefore" in the middle of a sentece and rather replace it by „but" or start a new sentence with „Therefore".

C10: Please, do not use short and long dash randomly, it has its rules. The „long" dash is usually used for a range of values, wherase the „short" dash is usually used as a word connector. On page 47, you use both types of dashes for the same purpose (a range span), this is really eye-striking!

I have no doubt that the candidate is ready to defend her thesis and recommend this whole-heartedly.

In České Budějovice, 10.6.2019

Jan Perner, PhD