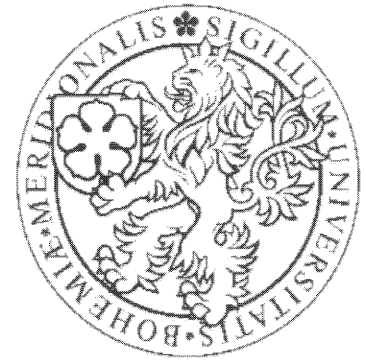


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Mentor's assessment of thesis by Marion Sieber: **Protein-protein interaction of photoperiodic clock factors in *Pyrrhocoris apterus***

Marion started to plan her master project approximately one and half years ago. She actually started, due to some exams and so on a few months later, having exactly one year to do all the experiments. Thematically, her project follows up our genetic evidence of interaction between and among several transcription factors. These factors include classical circadian proteins, but also recently identified receptor of Juvenile hormone Met and its partner Tai. The actual physical interaction was elusive, at the same time important for our further research and really interesting for this chronobiology field. There are no good antibodies for these proteins, and our insect model species is beautiful for its biology, but it is not user friendly model. Therefore we were looking for a quick and relatively high throughput approach, such as yeast two hybrid assays. And this seemed as optimal master thesis project.

The dream version of the project was following: Generate clones for the most interesting bHLH proteins and test interaction of resulting proteins in yeast and also elucidate the impact of JH (using its mimics) on these interactions. Having these results in hand the next step would be generation of modified proteins – e.g. short deletions, substitutions, or modifications of JH binding pocket (which was recently identified). Given the relatively short amount of time, the optimal version simply was not possible. Therefore I decided to add a few more candidates to the study, which might reveal some interesting interactions and allow to finish the thesis in a shorter amount of time.

Marion managed to identify, amplify and clone open reading frames (orf) of a several proteins, some of them being expressed at very low levels. After sequence verification, these orfs were shuffled into either prey or bait yeast expression constructs. Then Marion managed to perform first rounds of yeast two hybrid experiments. Some of interactions were also tested in presence of Juvenile hormone mimicking compound. Clearly, this is remarkable amount of work. Obviously, most of these assays need to be repeated, but with existing plasmids it can be done quite efficiently. Despite certain technical imperfections, which look worse on pictures, certain interesting interactions were suggested and are worth of following up by appropriate alternative approaches.

In case of Marion I would particularly highlight her ability to think about experiments, and ability to write independently. She perhaps struggled with the biological consequences and background a little bit, which we might expect from chemist. It is also apparent in some regions of the thesis. However, taken together, I think that the work exceeds requirements for master thesis and I am happy to recommend the thesis for defense.

Last, but not least I would like to specifically thank Olina Bazalova for supervising Marion during all experiments, troubleshooting and time devoted to the project and I would like to wish Marion good luck in her future scientific, chemical and personal life.

České Budějovice
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