



Přírodovědecká  
fakulta  
Faculty  
of Science

Jihočeská univerzita  
v Českých Budějovicích  
University of South Bohemia  
in České Budějovice

## STATEMENT OF THE BACHELOR/~~DIPLOMA~~<sup>\*</sup> THESIS SUPERVISOR

Name of the student: **Andrea Hauserová**  
Study program: **Biology**  
Department/Institute: **Department of Molecular Biology & Genetics, Faculty of Science.**  
Thesis title: ***Analyzing the role of p38 mitogen activated protein kinases and their effectors on mouse blastocyst maturation.***  
Supervisor: **doc. Alexander W. Bruce, Ph.D.**  
Supervisor`s affiliation: **Faculty of Science, University of South Bohemia**

	Point scale <sup>1</sup>	Points
<b>(1) FORMAL REQUIREMENTS</b>		
Formal and graphical quality of the thesis	0-3	3
Ability to work with literature	0-3	3
Language and stylistics	0-3	3
Formal requirements – points in total		9
<b>(2) PRACTICAL REQUIREMENTS</b>		
Fulfilment of the aims	0-3	3
Ability to understand the results, their interpretation, and clarity of the results, discussion, and conclusions	0-3	3
Discussion quality – interpretation of results and their discussion with the literature	0-3	1
Logic in the plan of the experimental work	0-3	3
Experimental difficulty of the thesis, independence in experimental work	0-3	3
Contribution of the thesis to the knowledge in the field and the possibility to publish the results (after eventual supplementary experiments)	0-3	3
Practical requirements – points in total		16
<b>POINTS IN TOTAL (MAX/AWARDED)</b>	<b>27</b>	<b>25</b>

Overall classification: 3-excellent

\* Choose one

<sup>1</sup> Mark as: 0-unsatisfactory, 1-satisfactory, 2-average, 3-excellent.

### **Eventual additional comments of the supervisor on the student and the thesis:**

It was my pleasure to have Andrea working in our research group. She was/ is very well liked by myself and other members of the laboratory (especially with the added bonus of her exceedingly good and creative cake making skills!). During her time in the laboratory Andrea proved herself to be a very diligent and careful worker and more than met my expectations of what is required of a Bachelors' level research student (contributing data to two manuscripts, that at the time of writing, are under consideration at the journals *Communications Biology* and *Open Biology*, with 2019 impact factors of 4.165 and 4.931, respectively). Andrea also presented her draft thesis well in advance of the submission deadline and responded very well to the suggestions provided by myself and her day-to-day laboratory co-supervisor.

Without wishing to pre-empt the contents of Andrea's thesis and defence presentation, she worked in very close collaboration with my senior Ph.D. student, Pablo Bora (finishing his 4<sup>th</sup> year of studies on the role of p38-MAPKs during mouse blastocyst ICM cell fate derivation and the specification of primitive endoderm in particular), and assisted him with both analyses of data already acquired by him and in the derivation of new data, by active bench based experimentation, that she also analysed. As mentioned, these data have been incorporated into two submitted manuscripts that together with an already published paper will form the backbone of Pablo's Ph.D. thesis defence. It is important to note that whilst I myself was in overall supervision of these experiments/projects, it was Pablo Bora that performed the majority of day-to-day supervision of Andrea (including thesis preparation, that I consider an important component of his Ph.D. training).

Andrea was thus engaged in assaying phenotypes related to primitive endoderm specification during mouse blastocyst embryo maturation related to p38-MAPK signalling (previously identified as a contributing factor in our laboratory). Using previously acquired confocal micrographs of embryos cultured in  $\pm$ p38-MAPK pharmacological inhibited conditions, she confirmed p38-MAPK inhibited derived blastocysts not only presented with PrE specification defects but also had statistically significant reductions in cavity volumes. This resonated with a recent report implicating expanding blastocyst cavity volume as a significant contributing factor to primitive endoderm derivation (and was included in the *Communications Biology* submission). Secondly, she actively assayed, via confocal based immuno-fluorescent microscopy, the protein expression of an identified p38-MAPK effector/substrate, DDX21, during blastocyst maturation and showed its expression to shift from nuclear to exclusively nucleolar as the blastocyst matures and the primitive endoderm specifies. She showed this relocalisation is sensitive to p38-MAPK inhibition. Moreover, analysing confocal micrograph images of late blastocysts containing marked RNAi-mediated *Ddx21* knockdown clones (generated by Pablo), she was able to confirm both cell autonomous (within the clone) and non-autonomous (outside the marked clone) reductions in DDX21 protein expression and significantly reduced derivation of inner-cell-mass lineages, particularly the primitive endoderm (data included in the *Open Biology* submission).

Thus, Andrea's work involved learning to collect and *in vitro* culture preimplantation stage mouse embryos under control and pharmacologically supplemented conditions, fix and immuno-fluorescently stain blastocysts. Moreover, obtain confocal embryo micrographs (or work with existing datasets) and analyse specific numbers of various cell types (*e.g.* blastocyst lineages), normalised specific protein expression levels (*i.e.* DDX21) and blastocyst cavity volumes (all with attendant statistical analyses). In this regard, Andrea demonstrated a very good laboratory and analytical technique.

Throughout Andrea's time in the laboratory, both Pablo and I were always confident that she fully understood both the theory and practice of what she was doing. Further illustrating this point, Andrea wrote a comprehensive and good quality Introduction regarding preimplantation mouse embryo development in her thesis (although her Discussion was a little brief, a common complaint

for most Bachelors level theses – but nonetheless it was relevant and to the point).

Overall, I was very satisfied with Andreas contribution to the laboratory. She has received a solid foundation in experimental design, execution, interpretation and presentation (that will hopefully result in her first two scientific papers as a researcher). I wish her good luck in whatever she next plans to embark upon. I have no hesitation in recommending Andrea’s Bachelors project thesis is accepted as successfully defended.

**Conclusion:**

In conclusion, I,

Alexander W. Bruce

~~recommend / do not recommend\*~~.

In: České Budějovice

Date: 14<sup>th</sup> April 2021

..... signature