



Supervisor's evaluation of Lucie Novotná's bachelor's project: "Investigation of the function and composition of the mitochondrial MRB1 complex in *Trypanosoma brucei*"

Lucie Novotná joined the Laboratory of Molecular Biology of Protists in 2006, at the beginning of her bachelor's studies at the Department of Molecular Biology. She soon came under my supervision on a project whose ongoing aim is to determine the function and composition of a putative mitochondrial (mt) RNA binding complex (MRB1), which was discovered to associate with an RNA editing accessory factor called TbRGG1. Because the role of MRB1 in the unusually intricate mt RNA metabolism of *Trypanosoma brucei* was unknown, the strategy for achieving this goal often changed as new data further refined our understanding of its function. This aspect of the project is probably apparent to readers of Lucie's thesis, which describes her key contributions via a wide spectrum of molecular and biochemical techniques. They are part of a bigger story, in which we hypothesize that this complex is involved in expression of small guide RNA genes encoded on the minicircles, which comprise a bulk of the mt genome of this eukaryote.

As written in her thesis, Lucie learned such methods as PCR, molecular cloning, transformation and cultivation of *T. brucei*, Northern blots, acquiring a very solid research background. Her main focus was the overexpression of two components of MRB1 called Zahada's 1 and 2. Despite her considerable efforts, the expression of these proteins was not as robust as needed for generation of antibodies. Thus, she experienced very early on an unfortunate part of science, when problems arise that simply do not yield to persistence or a sound and systematic approach. Instead of being discouraged, she kept trying, playing with



different conditions until we had an opportunity to generate the antibodies against manufactured oligopeptides. This step enabled her to continue to address the composition of the MRB1 complex by successfully employing difficult methods such as isolation of mitochondria and glycerol gradient separation of macromolecules by ultracentrifugation.

It became clear to me during Lucie's participation in this project that she understood how RNAi works in *T. brucei*, as well as starting to grasp RNA editing and the unique nature of mt DNA in kinetoplastids, topics that are quite baffling to researchers further along in their careers. She has proven to be perseverant, motivated methodological and willing to learn new techniques. Her willingness to collaborate also has helped to make the ambiance we have within our small working group-and the laboratory as a whole-much more pleasant and fun. It has been my pleasure to supervise Lucie's thesis and I look forward to working with her during her masters studies.

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Mgr. Hassan Hashimi