

Molecular Parasitology

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Thesis Review

Doctoral thesis submitted by Nadine Zimmann MSc., entitled: "Analysis of lysosomes of *Trichomonas vaginalis*"

The thesis submitted by Nadine Zimmann was designed to investigate the lysosomes of the parasitic protist *Trichomonas vaginalis*, lysosomes being the main degrading organelles in eukaryotic cells. The aims of her thesis were to develop and optimize a protocol for the isolation of lysosomes from *T. vaginalis*, to analyse their proteome, to elucidate the role of N-linked glycosylation in lysosomal protein targeting, to investigate the role of lysosomes in hydrolase secretion and to analyse the secretome of *T. vaginalis* for lysosome-derived virulence factors. Nadine Zimmann together with co-workers validated Rab7a as a late endosomal/lysosomal marker in *T. vaginalis*. They found a magnetic bead-based approach to result in the optimal isolation method for lysosomes and revealed a lysosomal proteome of *T. vaginalis* of 462 proteins in 21 functional classes, with hydrolases representing the largest class. Moreover, they identified six transmembrane proteins that are homologues to the mammalian MPR300, but based on their work the MPRs of *T. vaginalis* do not bind lysosomal cargo through M6P. Interestingly, they also provided evidence that the cysteine protease CLCP is targeted to lysosomes in a glycosylation-dependent manner. They also identified several hydrolases in the secretome, including the cysteine protease TvCP2. Finally, they revealed that the phagolysosomal proteome and the secretome of *T. vaginalis* overlap by 26 proteins and they found evidence that in *T. vaginalis* a broad range of hydrolases might in fact be unconventionally secreted. Altogether, the thesis of Nadine Zimmann significantly advances our understanding of lysosomes, not only in *T. vaginalis*. Her work suggests a deep evolutionary origin of lysosomes in eukaryotes, but also highlights differences that might be relevant for parasite virulence.

The thesis itself is clearly written and very well structured. In the Introduction, Nadine Zimmann gives a solid and detailed review on cellular trafficking and summarizes the background of the research question. She has performed an exhaustive literature review and presents the current state of knowledge on lysosomes, on endo and exolysosomal pathways

and on the lysosomal degradome and secretome with a special focus on *T. vaginalis*. Subsequently she describes the aims and specific objectives of her thesis. The following chapter summarizes the results and conclusions. She gives a comprehensive overview of the data obtained and discusses the relevance and significance of her data thereby also demonstrating her high expertise in the field. The data evaluation is scientifically sound and the results are very clearly presented. Finally, Nadine Zimmann presents the publications that have arisen from this thesis, thereby also commenting on the contributions and giving an overview of the abbreviations used and the references cited in her thesis.

This thesis has resulted in two publications, both published in the high impact international peer-reviewed scientific journal *Molecular and Cellular Proteomics* (IF: 5.911; Q1 in the category "Biochemical Research Methods"), one in 2018 and one in 2021, with Nadine Zimmann being the first author on the latter.

In conclusion, the doctoral thesis submitted by Nadine Zimmann is highly innovative, the experiments and data analyses performed are scientifically sound and the conclusions drawn are not only highly interesting and relevant to the scientific community and beyond, but also careful and convincing. Her work represents a significant step forward in understanding the function of lysosomes and their proteome and has resulted in two top publications. Altogether, I judge this thesis as excellent with distinction.

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Date



Signature