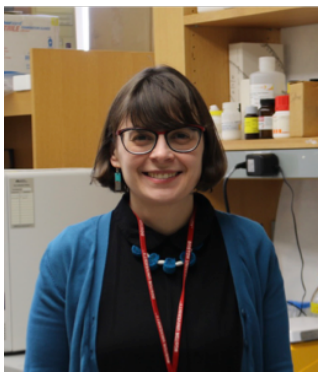
Principal Investigator: Giulia Bandini
Grant Title: Studies on the activity and function of *T. gondii* and *C. parvum* SPYs

Abstract



Toxoplasma gondii and *Cryptosporidium parvum* are protozoan parasites and the etiological agents of human diseases. *T. gondii* causes developmental defects in fetuses and neurological damages in immunocompromised individuals, while *C. parvum* causes severe diarrhea.

We previously described the presence of *O*-fucose on Ser/Thr residues of *T. gondii* nuclear proteins and showed by gene knock out that *Tg*SPY is the protein *O*-fucosyltransferase responsible for the modification. *Tg*SPY is an ortholog of the recently described *Arabidopsis thaliana O*-fucosyltransferase SPINDLY and a paralog of metazoan *O*-GlcNAc transferases (OGTs). The *C. parvum* genome also encodes for a SPY-like enzyme and preliminary results suggest the presence of nuclear *O*-fucosylated proteins in this parasite. However, previously published work biochemically characterized *Cp*SPY as an OGT.

The aim of this proposal was to further study *T. gondii* and *C. parvum* SPYs to learn more about the biochemistry of nucleocytosolic *O*-glycosyltransferases from the CAZy GT41 family.

In the first part of the project we complemented *T. gondii spy*-deficient parasites with either full-length *T. gondii*, *C. parvum* and *A. thaliana* SPYs or a set of *Tg*SPY mutants. Only complementation with the wild type endogenous enzyme completely restored binding by the fucose-specific *Aleuria aurantia* lectin and both *A. thaliana* and *C. parvum* SPYs were detected as truncated in *T. gondii* lysates, suggesting they are not correctly expressed by the parasite. Mutation to Ala of conserved residues in the GT41 catalytic domain resulted in either inactive enzyme or strongly reduced transferase activity. Finally, complementation with a truncated version of *Tg*SPY, which had only three tetratricopeptide repeats in the N-terminal domain, was not sufficient to restore AAL binding. In conclusion, complementation with *Tg*SPY point mutants confirmed the presence of conserved residues important for catalysis in all GT41 enzymes, wherever SPY- or OGT-like, suggesting there might be a common reaction mechanism independent of sugar nucleotide specificity.

The second part of the proposal aimed to further biochemically characterize *Cp*SPY activity *in vitro* and to identify *O*-fucosylated proteins in *C. parvum.* AAL enrichment followed by mass spectrometry analysis identified two putative Phe/Gly (FG)-repeat nucleoporins as modified with deoxyhexose(s). This observation is consistent with a model where all SPY-like enzymes are *O*-fucosyltransferases.