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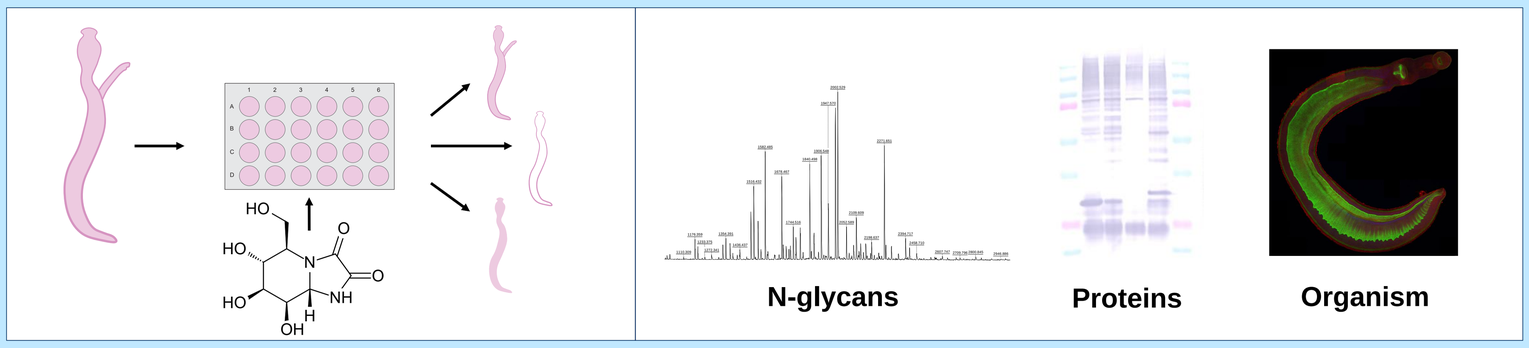
Glycoengineering of *ex vivo* cultured *Schistosoma mansoni* adult worms using chemical mannosidase inhibitors

Introduction

- Schistosomiasis is caused by *Schistosoma* parasites, affecting 250 million people worldwide. New drugs and vaccines are needed to prevent (re)infections.
- Adult worms can survive in a human host for decades, through the ability to **skew the host's immune response**.
- It is thought that the parasite uses **glycosylated molecules** for this skewing.
- Elucidating **which glycans** are involved, could help in the identification of new drug and vaccine targets.
- In this study, **we have developed glycoengineered adult worms as a tool for studying the impact of schistosome glycosylation on host-parasite interaction and parasite biology**.

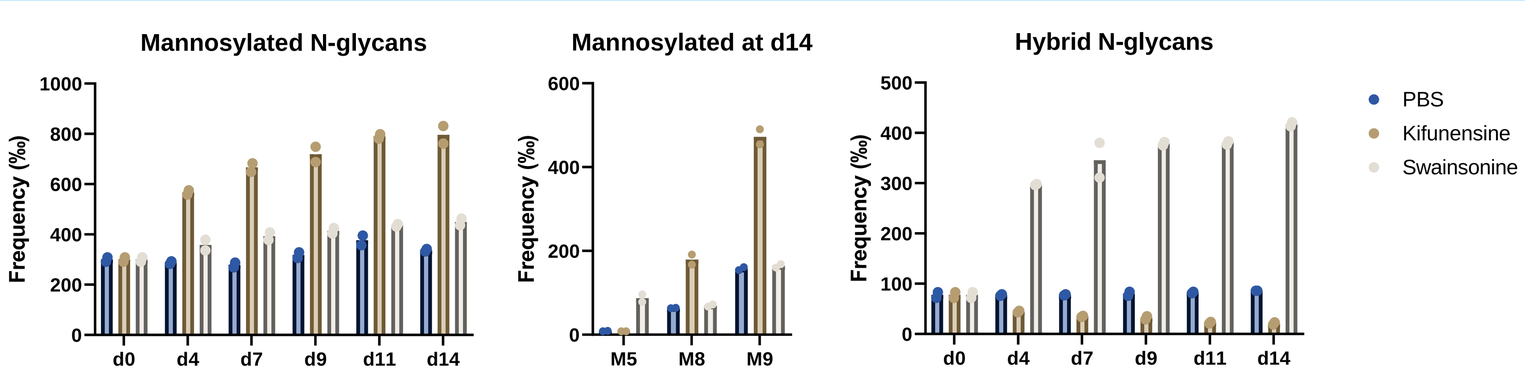
Experimental set-up

- *Schistosoma mansoni* adult worms were cultured *ex vivo* in presence of the chemical mannosidase inhibitors **kifunensine** (kif; 5 µg/mL) or **swainsonine** (swain; 25 µM) or phosphate-buffered saline (PBS) as mock-treatment for a maximum of fourteen days.
- **N-glycans** were isolated from homogenised worms with PNG-F, then purified and measured by MALDI-TOF MS.
- To analyse **glycoproteins**, homogenised worms were run over an SDS-PAGE, transferred to a membrane and incubated with glycan-specifc antibodies.
- Living worms were incubated with live dyes overnight, harvested and imaged with confocal microscopy to study **morphology**. Additionally, **motility** was assessed through WHO-TDR scoring.



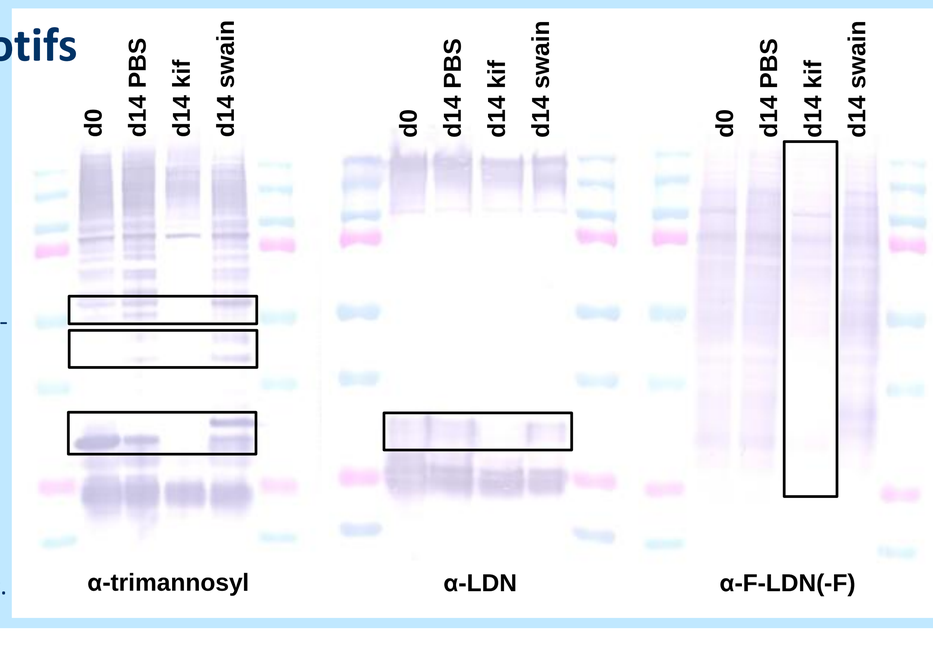
Effect of kifunensine and swainsonine on N-glycan profile

- Kif-treated worms showed an increase of mannosylated N-glycans (796‰ at d14), specifically N-glycans with eight (M8) or nine (M9) mannoses.
- Swain-treated worms demonstrated a rise of hybrid structures (417‰ at d14) and the "precursor" structure M5.



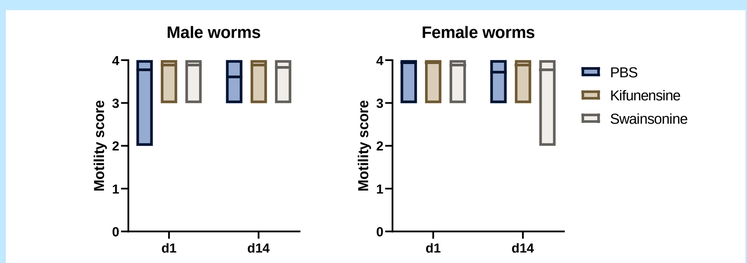
Presence of specific glycan motifs

- Protein preparations of uncultured (d0), mock-treated (d14 PBS) and glycomanipulated (d14 kif & d14 swain) adult worms were tested for the presence of three different glycan motifs.
- Trimannosyl, as present in mannosylated glycans (M3 & M5), were reduced in kif-treated worms and were expressed on different proteins in swain-treated worms.
- LacdiNAc (LDN), a motif specific for complex N-glycans, was less detected in a protein subset of kif-treated worms.
- The complex fucosylated Lewis X (LeX) and LDN-fucose (LDN-F) were reduced in kif-treated worms.

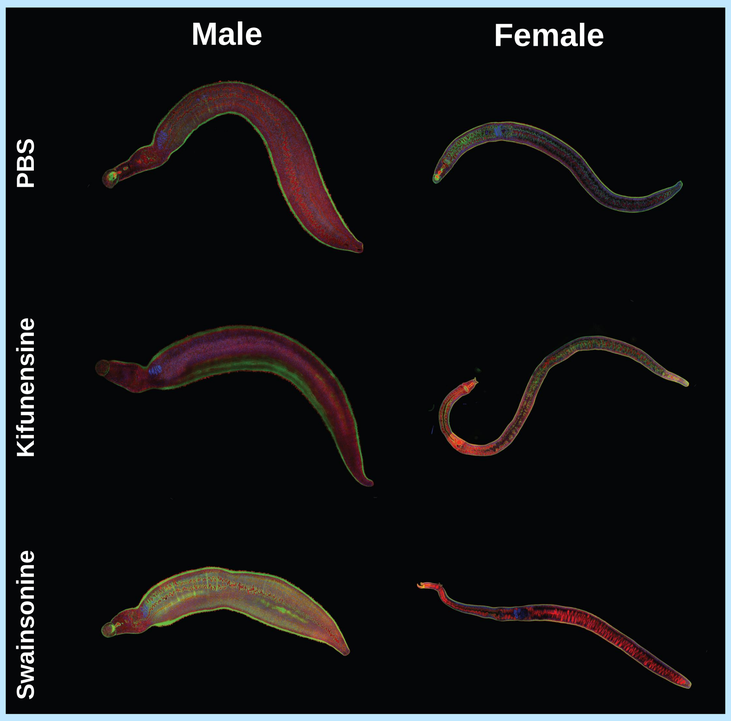


Motility and morphology of glycoengineered worms

- Kif- and swain-treated worms did not show impaired movement as compared to PBS-treated worms at d14.



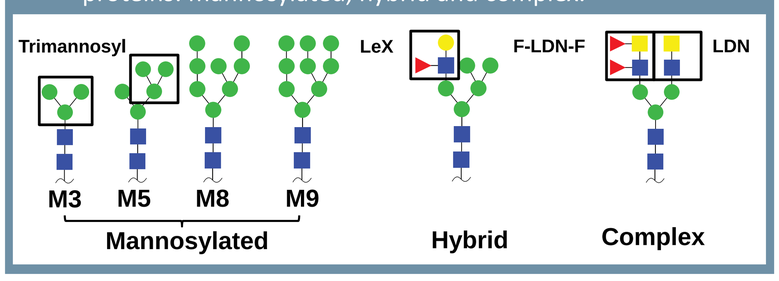
- Morphological features such as suckers, reproductive organs and tegument seemed unaltered in kif- and swain-treated worms when compared to PBS-treatment at d14.



Adult worms stained for nuclei (blue), membranes (red) and actin (green).

Glycans

- There are three main types of N-glycans, all attached to proteins: mannosylated, hybrid and complex.



Conclusions & future steps

- **The N-glycosylation profile of adult worm schistosomes can be altered through the use of chemical mannosidase inhibitors, resulting in an increase of mannosylated (kifunensine) or hybrid (swainsonine) glycans.**
- **The changes in N-glycosylation can also be detected at protein level.**
- ***Ex vivo* glycomanipulation with kifunensine and swainsonine for fourteen days does not lead to an impaired motility or altered morphology of adult worm schistosomes.**
- **In future research, the effects of glycoengineered worm-extracts on host immune cells will be studied.**

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