

Gut microbial β -glucuronidases in androgen reactivation and health implications

Background

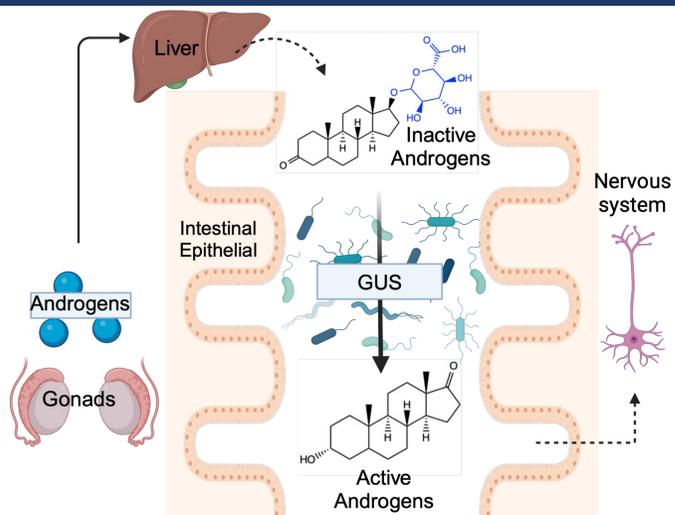


Figure 1. A schematic overview of androgen reactivation via the microbial enzyme, β -glucuronidase (GUS). Androgen dysregulation is implicated in polycystic ovarian syndrome (PCOS), prostate cancer, and irritable bowel syndrome (IBS).

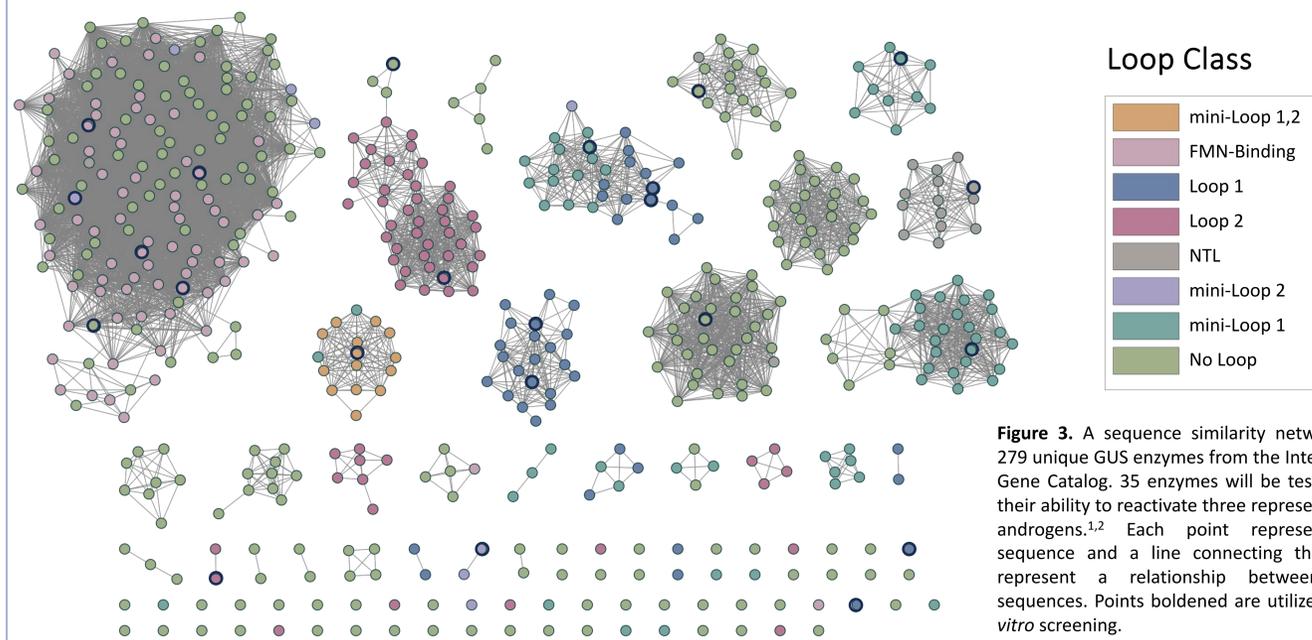


Figure 3. A sequence similarity network of 279 unique GUS enzymes from the Integrated Gene Catalog. 35 enzymes will be tested for their ability to reactivate three representative androgens.^{1,2} Each point represents a sequence and a line connecting the dots represent a relationship between the sequences. Points bolded are utilized in *in vitro* screening.

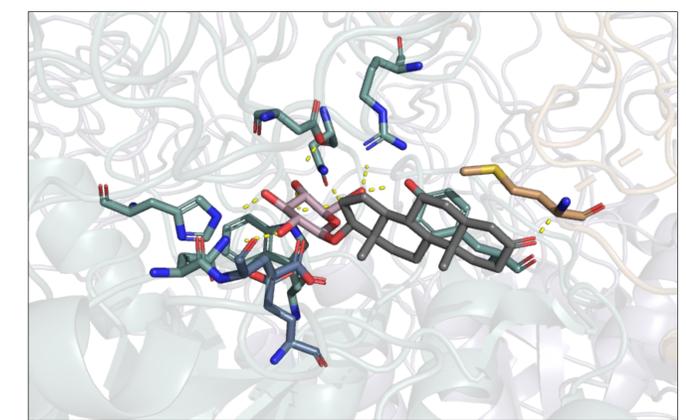
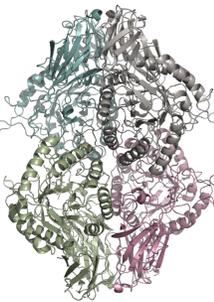


Figure 6. Preliminary docking studies indicate that the loop 1 region on the adjacent monomer (pale yellow) will influence the processing of the androgen-glucuronide (mauve and grey) in the primary monomer (teal). Further docking studies and subsequent mutations will need to be studied to confirm this hypothesis. The *E. eligens* protein used is PDB: 6BJQ docked with Maestro's Schrodinger software.

Conclusions

- Loop 1 and FMN-binding GUS enzymes show strong androgen-glucuronide turnover activity.
- Docking studies suggest the Loop 1 region influences androgen processing.
- E. coli* and *R. gnavus* are possible key enzymes in androgen reactivation.



Functional Diversity of GUS

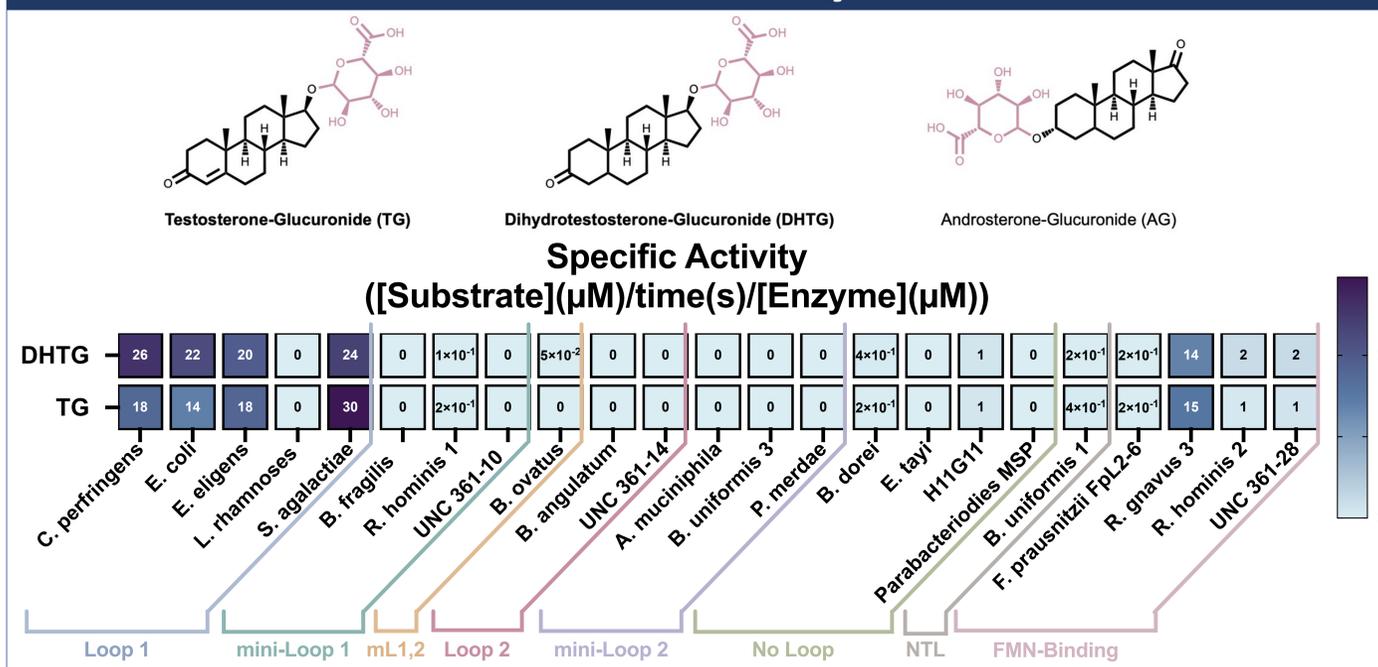


Figure 4. Specific activity assays allow for the comparison of each loop class's ability to process an androgen-glucuronide. Loop 1 GUSs and FMN-Binding GUSs process androgen-glucuronides compared to their counterparts. N = 3 biological replicates.

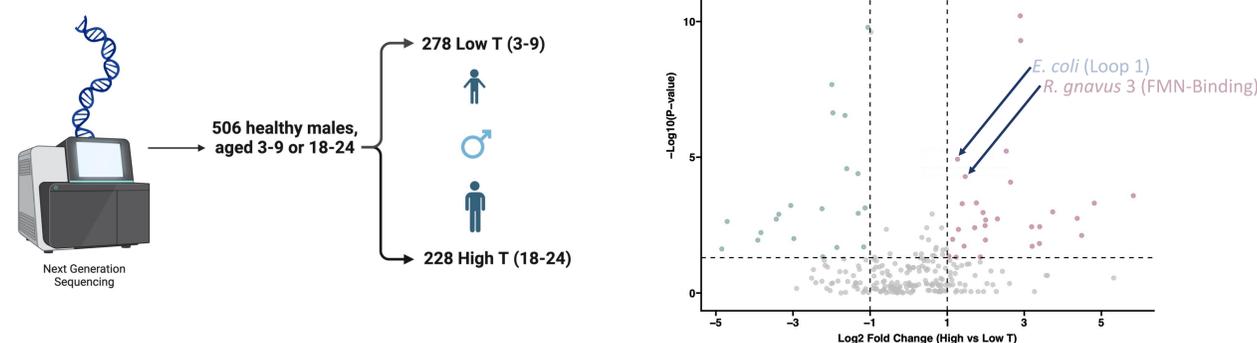


Figure 5. Upon investigating next generation sequencing, GUS enzymes that were upregulated in high-T states were found to match the specific activity *in vitro* studies.

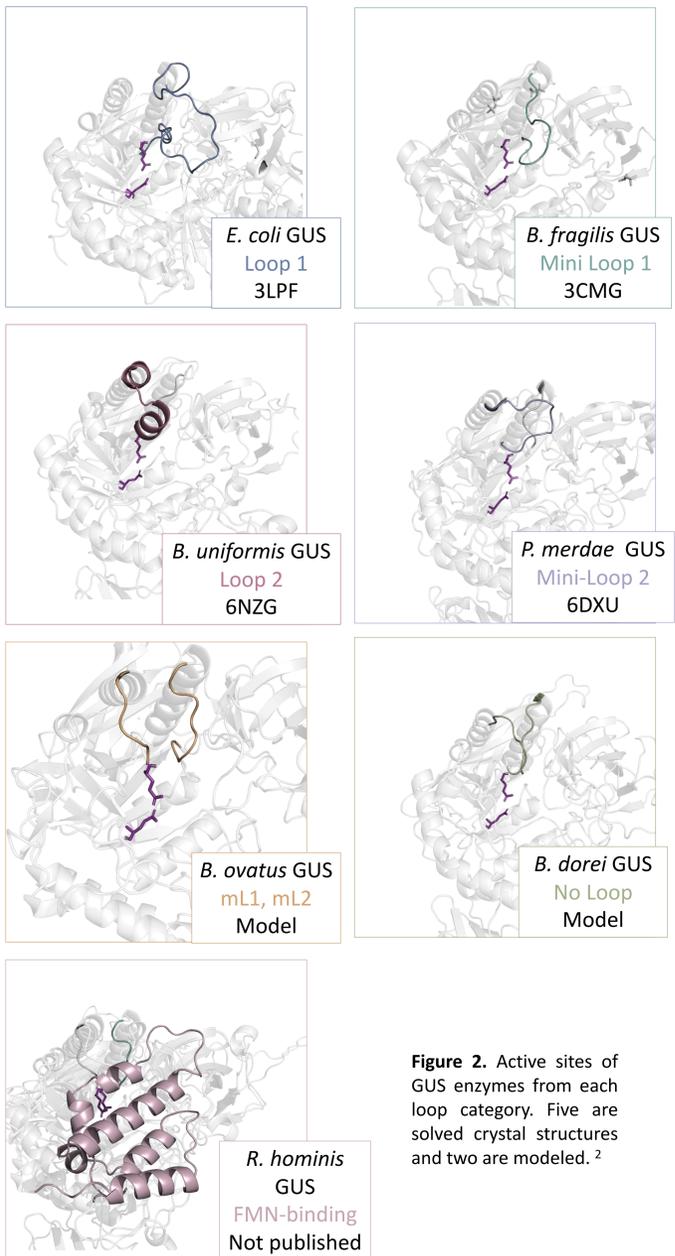


Figure 2. Active sites of GUS enzymes from each loop category. Five are solved crystal structures and two are modeled.²

Future Studies

- Specific activity assays with androsterone-glucuronide
- Docking and subsequent point mutation studies to confirm structural features involved in androgen reactivation.
- in fimo* assays with human stool samples combined with activity-based probe proteomics to identify microbial enzymes involved in androgen reactivation.

Acknowledgements

- We acknowledge current and previous Redinbo Lab members for helpful conversations.



References

- Ervin, S., et al *J. Biol. Chem.* 2019
- Pollet, R., et al *Structure* 2017
- Foley, M., et al *Nature Microbiology* 2023