



THE UNIVERSITY
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at CHAPEL HILL

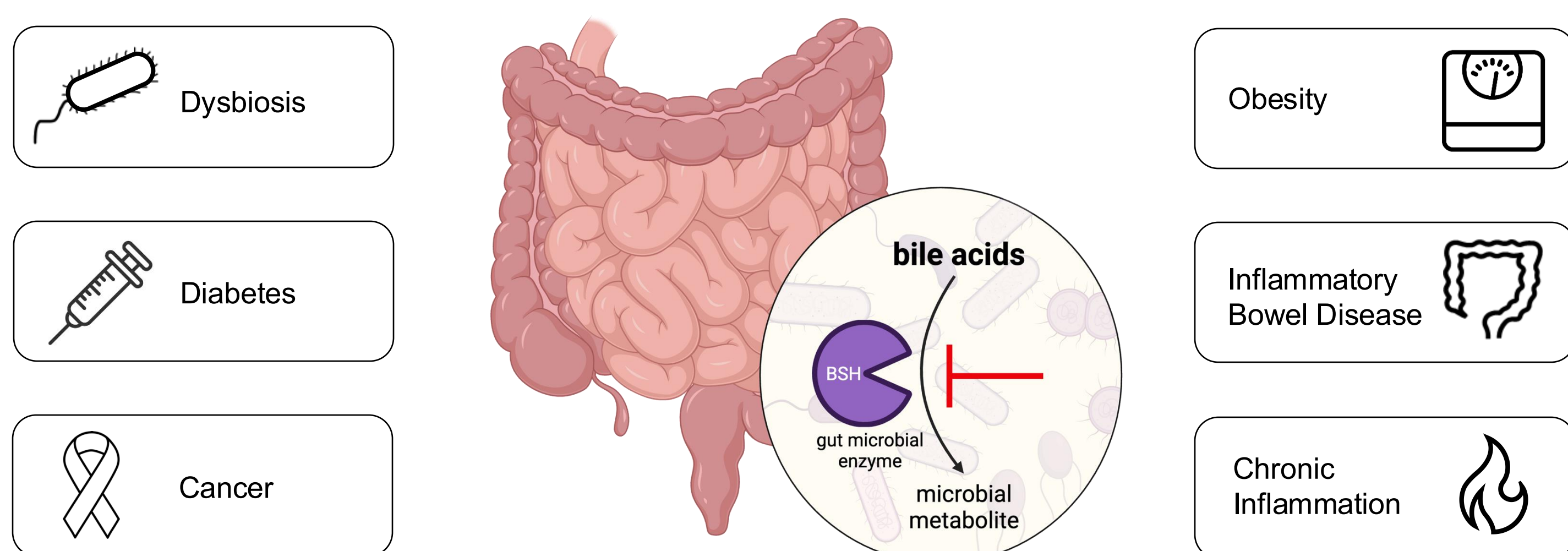
Investigating Gut Microbial Bile Salt Hydrolase Structure and Function with Activity-Based Probes and Inhibitors



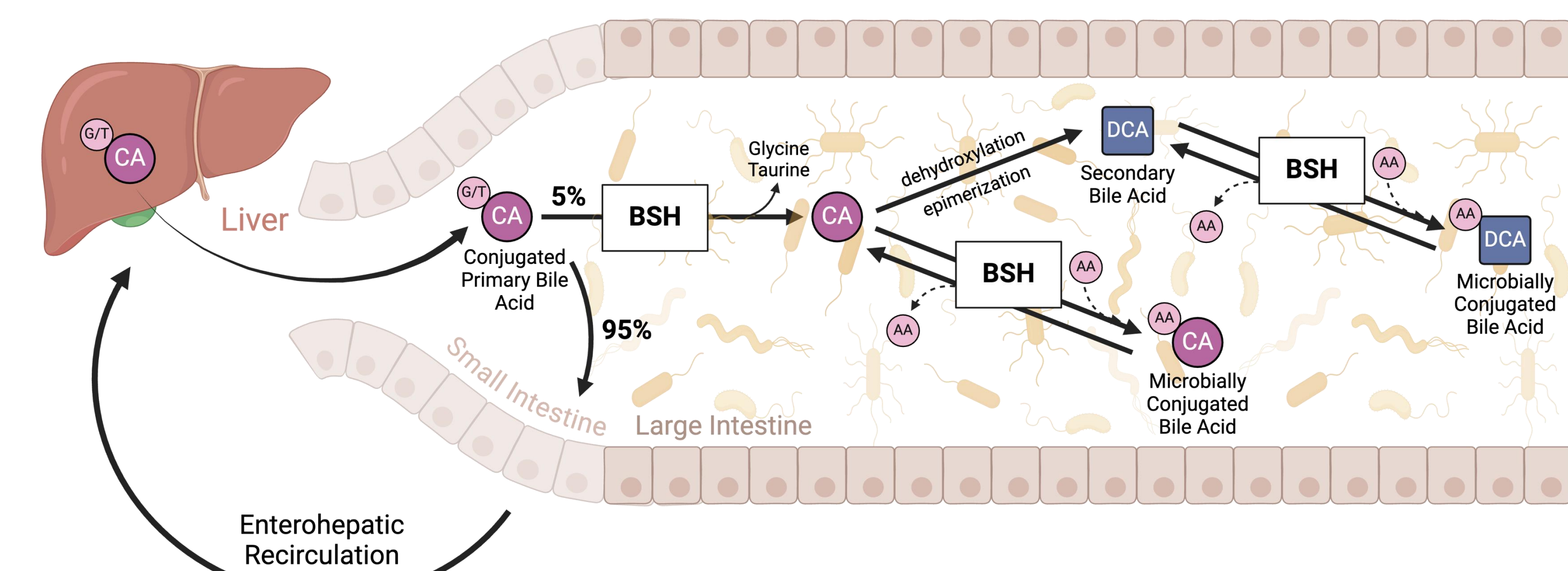
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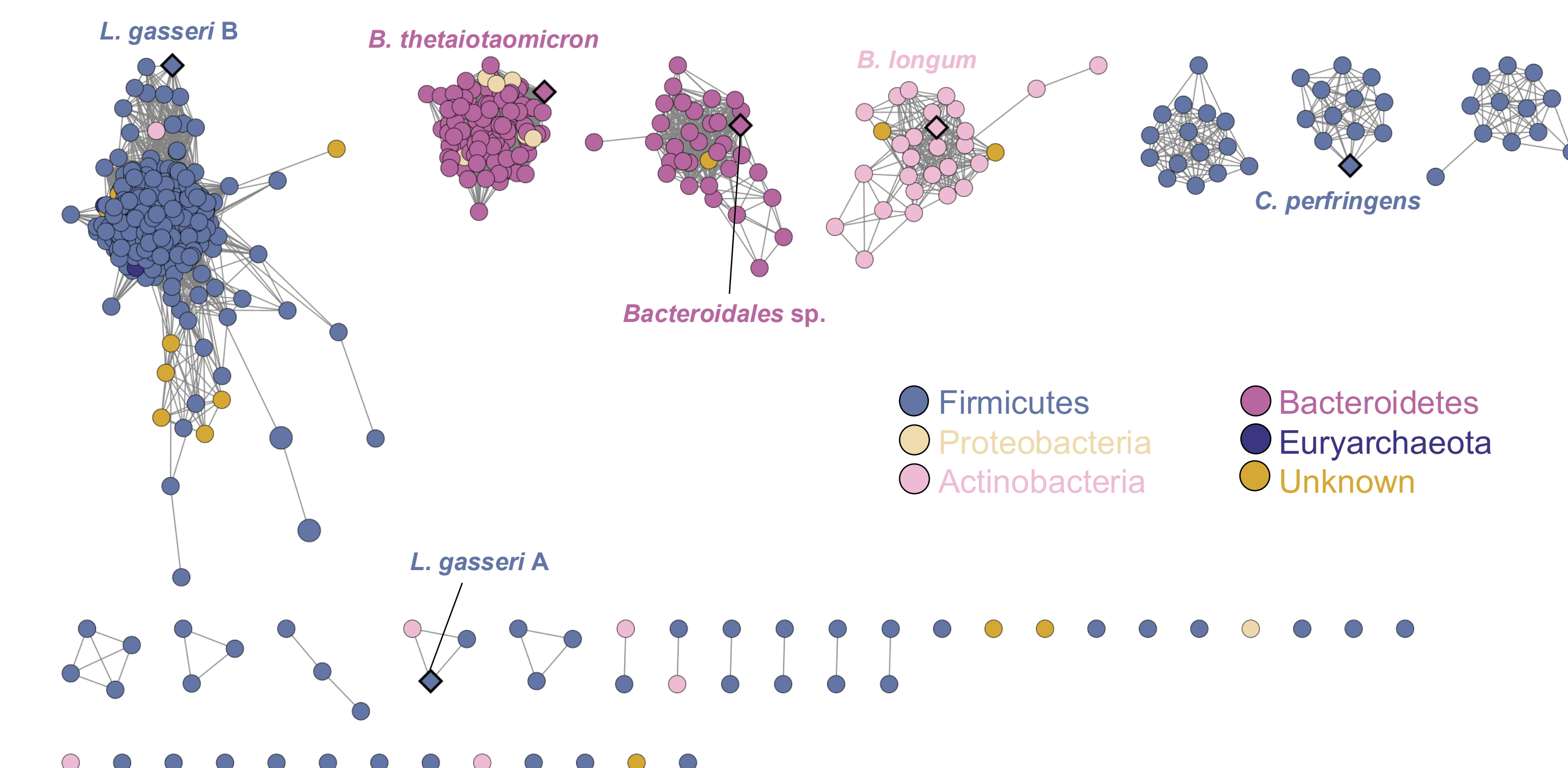
BILE ACIDS INFLUENCE HUMAN HEALTH



MICROBIAL MODIFICATION OF BILE ACIDS



SURVEYING BILE SALT HYDROLASE (BSH) DIVERSITY



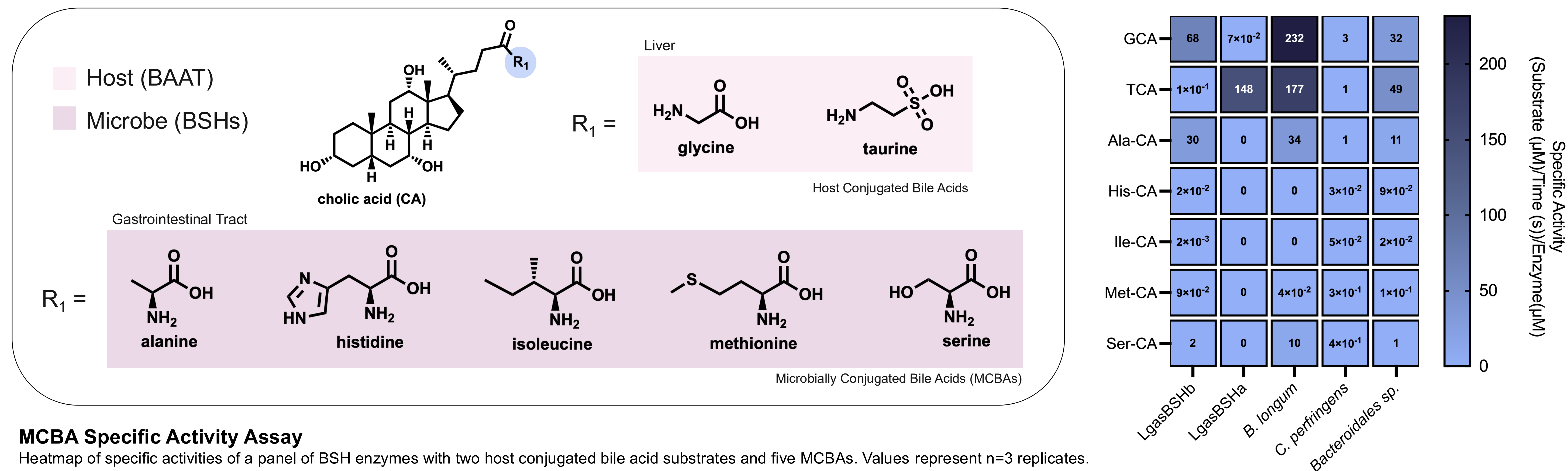
Sequence Similarity Network

BSH sequences were mined from the Integrated Gene Catalog using a rubric based on 5 key conserved residues in the active site and extant structures. 689 sequences were clustered by 95% sequence identity to yield 654 unique nodes (circles). The SSN was created using the EFI-EST tool and an E value of 1×10^{-100} . Bolded diamonds represent proteins selected for activity characterization.

REFERENCES & ACKNOWLEDGMENTS

We thank current and previous Redinbo lab members for all related intellectual discussions. [References](#): Foley *et al.* Nat Microbiol. 2023; Gangadoo *et al.* Int J Mol Sci. 2021; Adhikari *et al.* Nat Chem Biol. 2020; Adhikari *et al.* ACS Chem Bio. 2021.

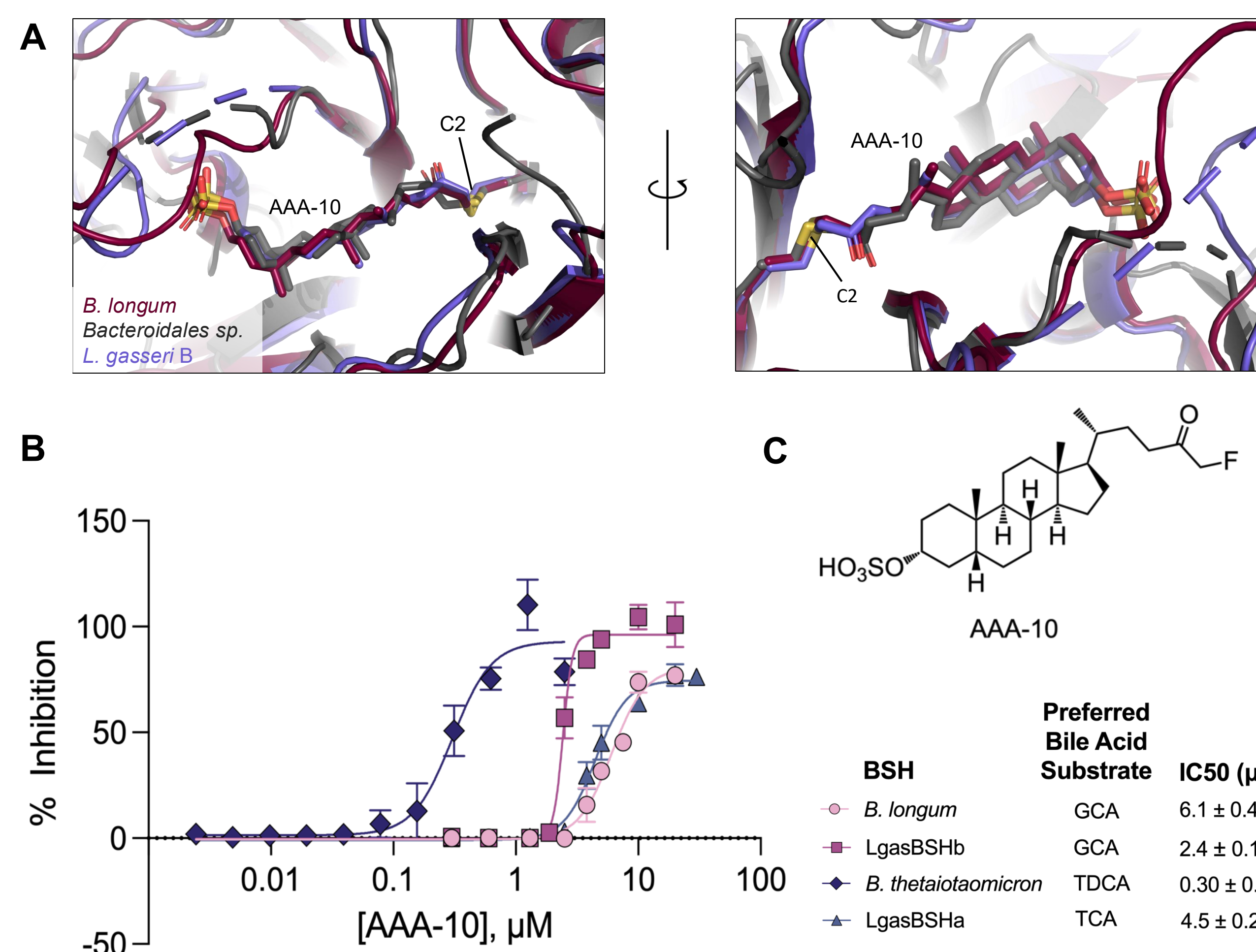
BSHs EXHIBIT PREFERENCE FOR HOST CONJUGATED BILE ACIDS OVER MCBAs



MCBA Specific Activity Assay

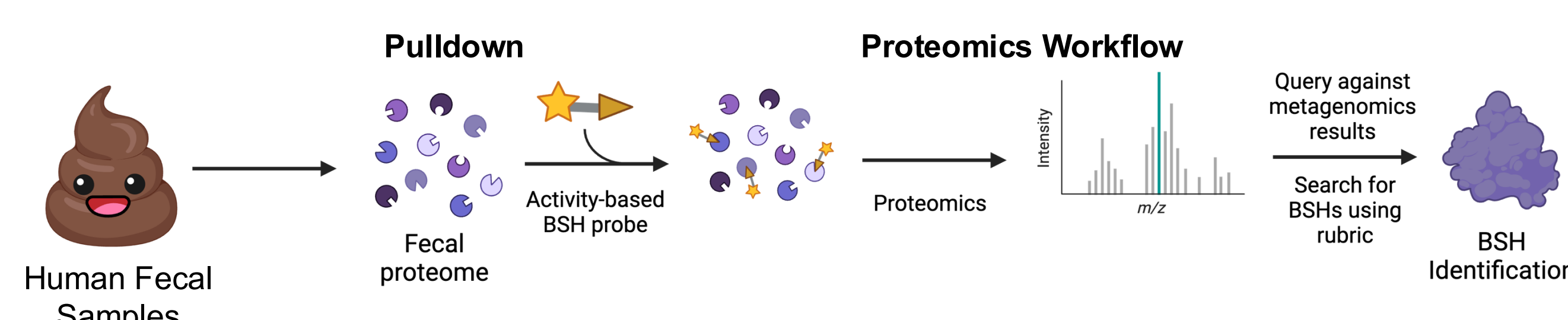
Heatmap of specific activities of a panel of BSH enzymes with two host conjugated bile acid substrates and five MCBAs. Values represent n=3 replicates.

AAA-10 INHIBITS DIVERSE BSH ENZYMES

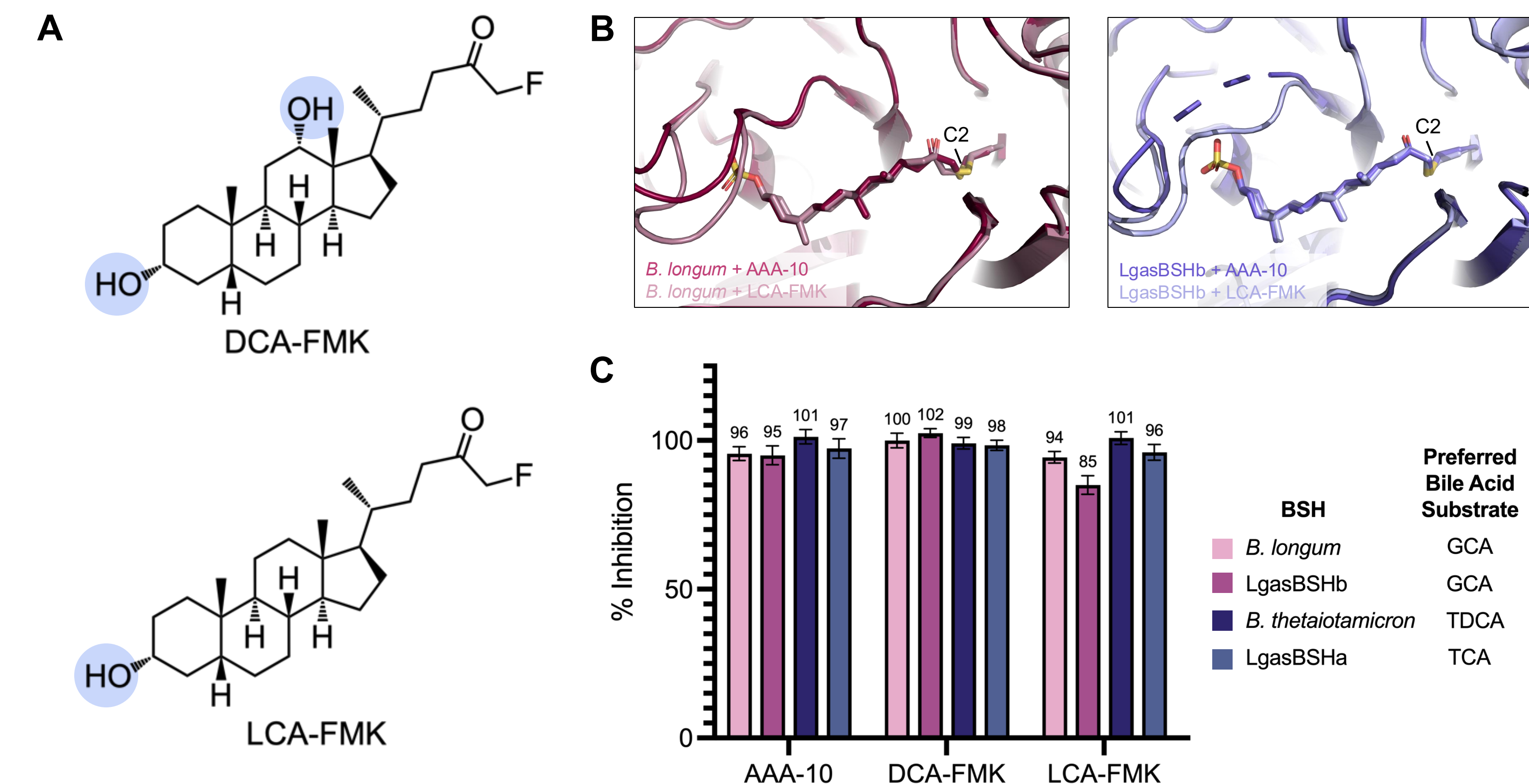


(A) AAA-10 has a similar binding mode to *B. longum* BSH (maroon), *Bacteroides sp.* BSH (grey), and LgasBSHb (purple). (B) AAA-10 IC₅₀ curves with a panel of four BSH enzymes and their preferred bile acid substrates. Potency ranges from 0.3 to 6.1 μM. (C) AAA-10 is a pan BSH inhibitor that contains a bile acid core, a fluoromethyl ketone warhead, and a sulfate moiety.

FUTURE DIRECTIONS

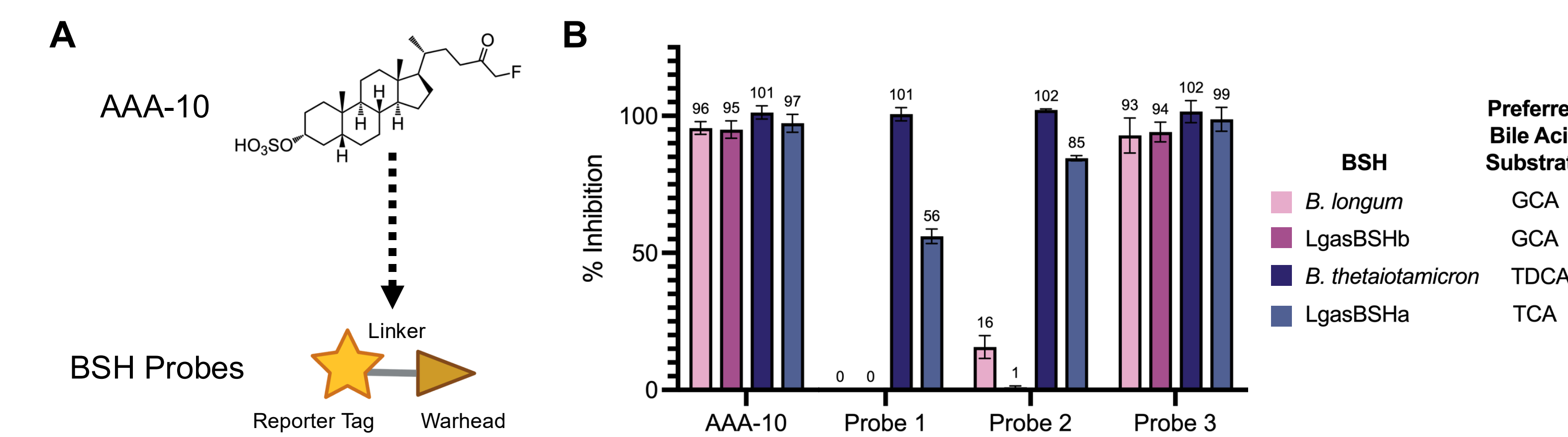


SIMILAR INHIBITION WITH DIFFERENT BILE ACID CORES



(A) Additional BSH inhibitors with varied bile acid cores. (B) LCA-FMK, the non-sulfated form of AAA-10, has the same binding mode at AAA-10 to *B. longum* BSH and LgasBSHb. (C) Inhibition of four BSH enzymes by AAA-10, DCA-FMK, and LCA-FMK with their preferred substrate at 20 μM concentration after a 4-hour incubation.

ONE BSH PROBE INHIBITS DIVERSE BSH ENZYMES



(A) AAA-10 was modified to create several activity-based BSH probes. (B) Inhibition of four BSH enzymes by the probes with their preferred substrate at 20 μM concentration after a 4-hour incubation.