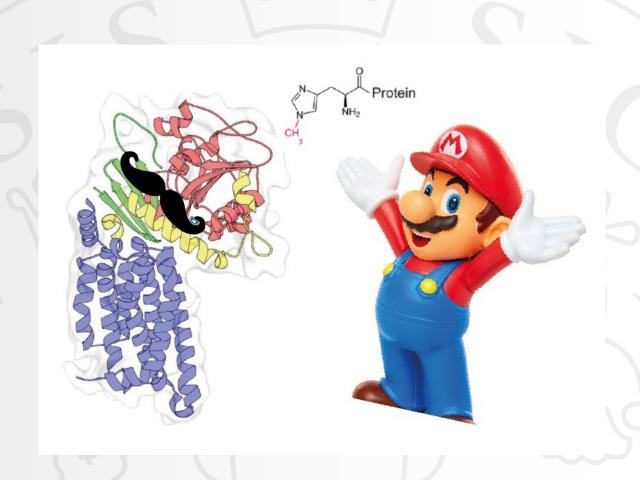
Identification of a fungal N-terminal histidine methyltransferase using targeted proteomics

Skyline user meeting ASMS 2024

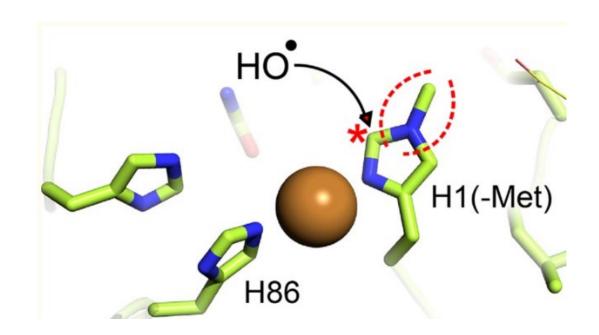
Tanveer S. Batth

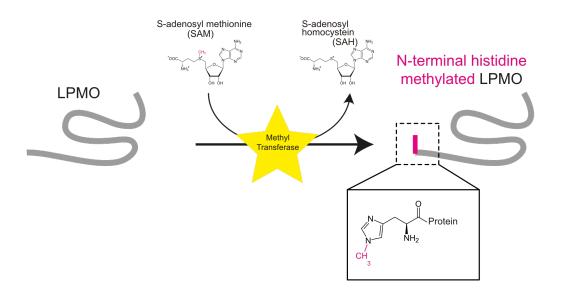


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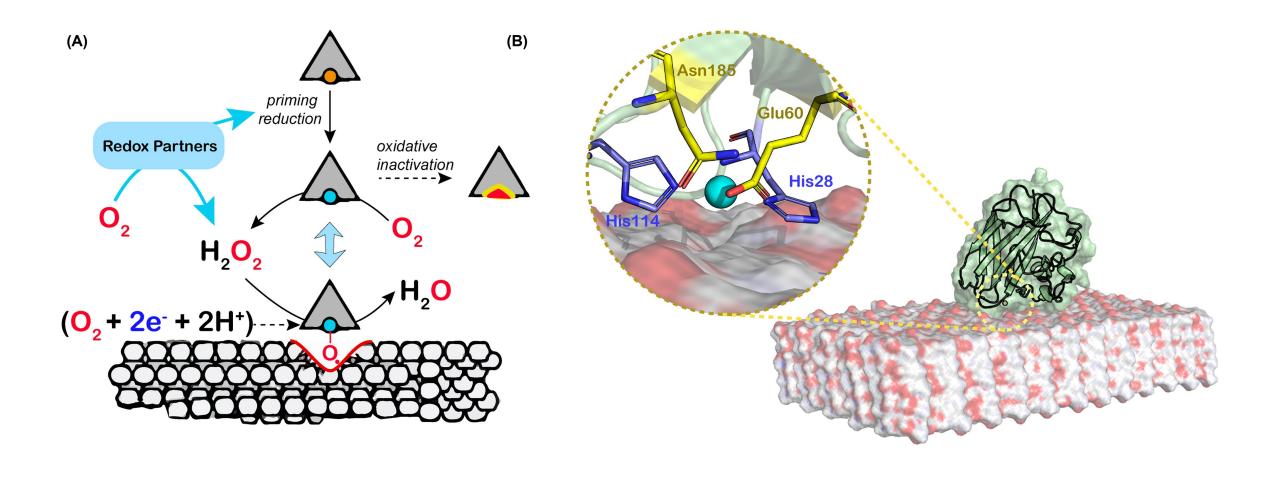


N-terminal methylation of fungal LPMOs

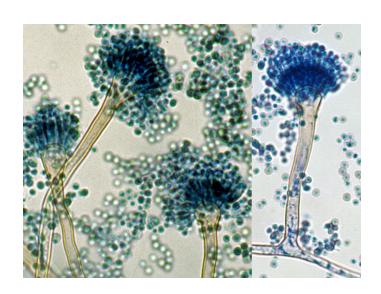




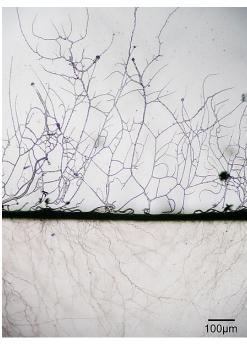
Lytic polysaccharide monoxygenases (LPMOs)



Filamentous fungi model organism

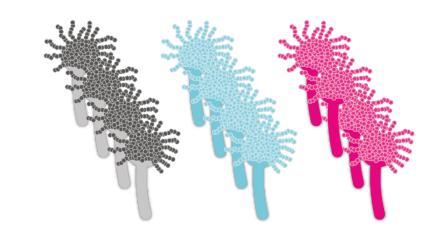


https://www.adelaide.edu.au/mycology/



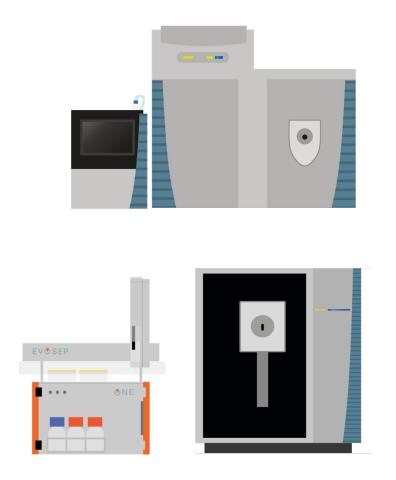
https://commons.wikimedia.org/

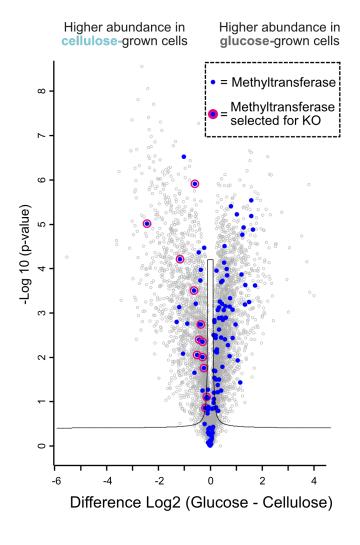
Aspergillus nidulans grown on glucose, cellulose, or potato dextrose (glucose)





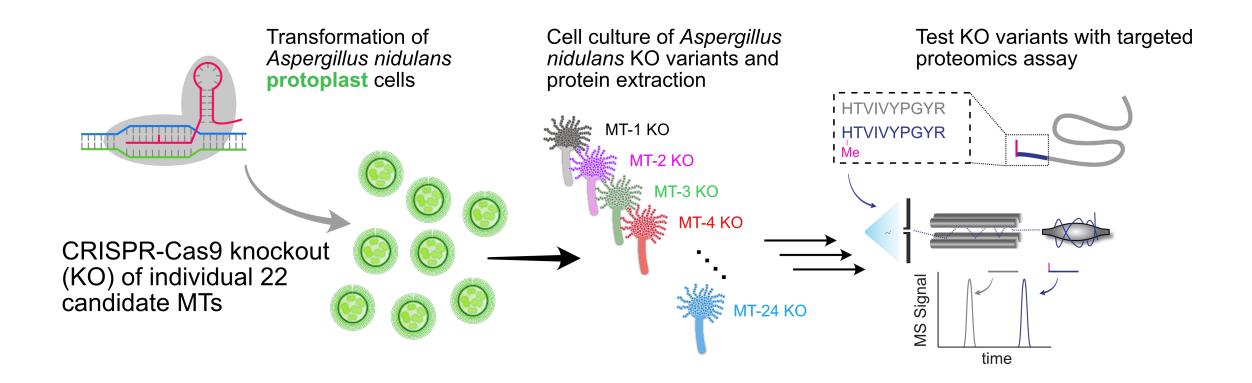
Discovery to Targeted – Two sides of the same mass spectrometer



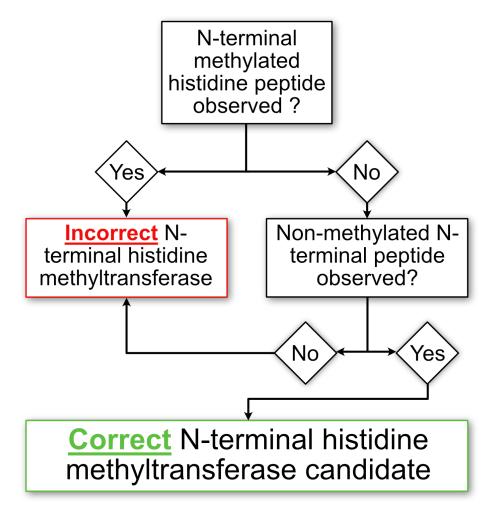




CRISPR/Cas9 knockout and proteomics strategy for identification of the NHMT



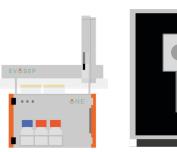
CRISPR/Cas9 knockout and proteomics strategy for identification of the NHMT

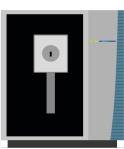


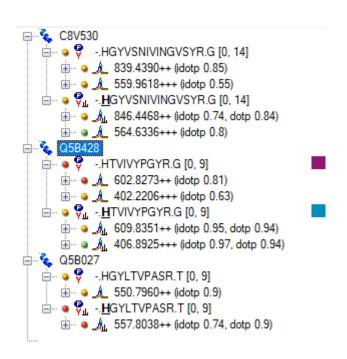


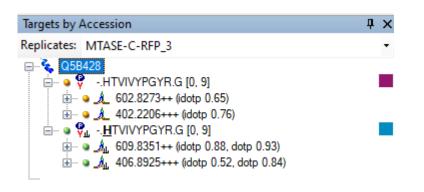
Discovery to Targeted – Establishing a Skyline method

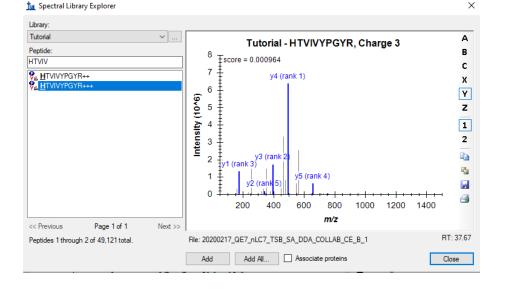




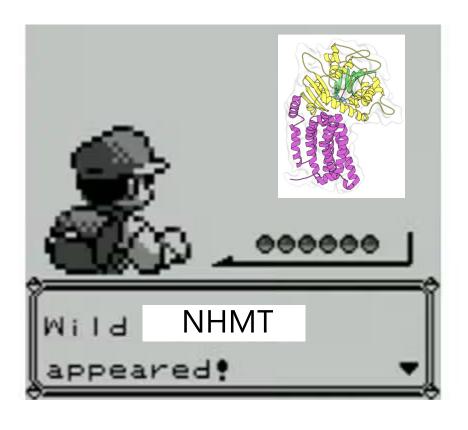


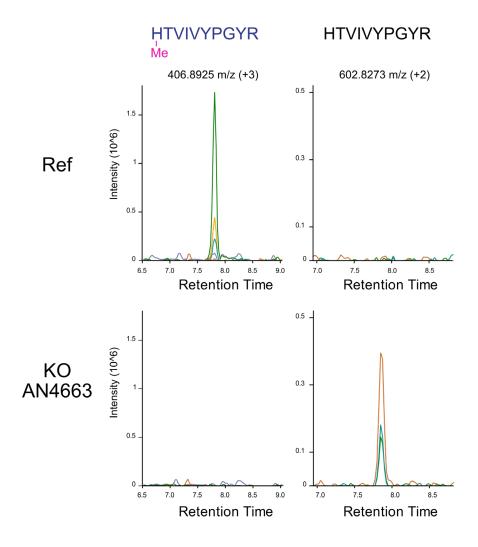






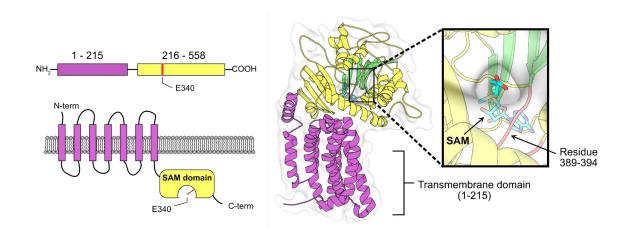
We found a hit!

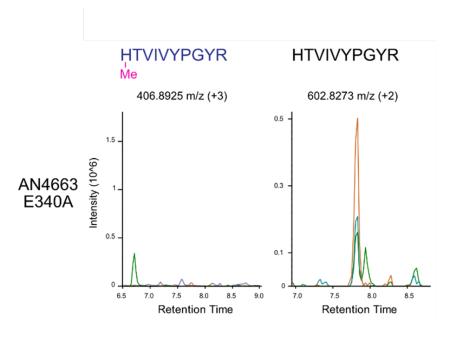






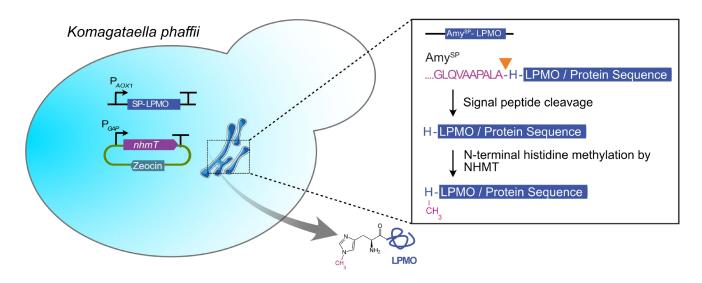
AN4664 – A novel transmembrane NHMT

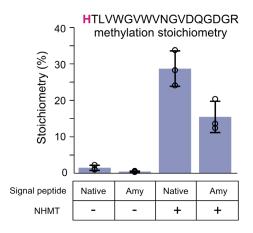


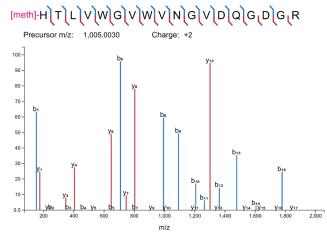


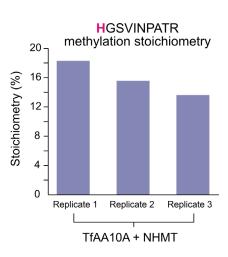


Production of novel N-term histidine methylated proteins in Komagataella phaffii



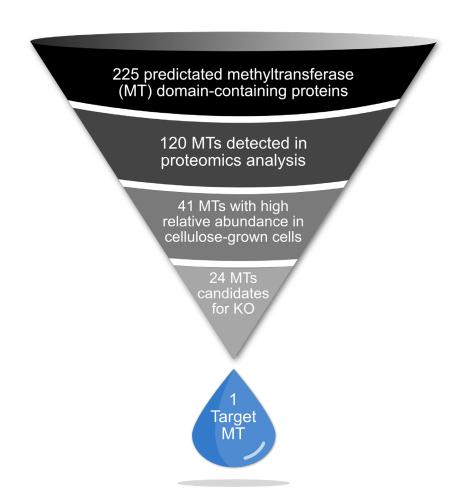






Summary

- Exploration of a novel PTM using a combination of discovery and targeted proteomics.
- Discovery proteomics to shortlist potential candidates.
- Targeted proteomics (powered by Skyline) assay to screen CRISPR/Cas9 knockout
- NHMT is a transmembrane protein which can be co-expressed in



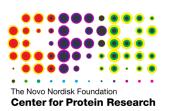
Acknowledgement

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- Jakob Hoof
- Søren Brander
- JVO + CPR colleagues









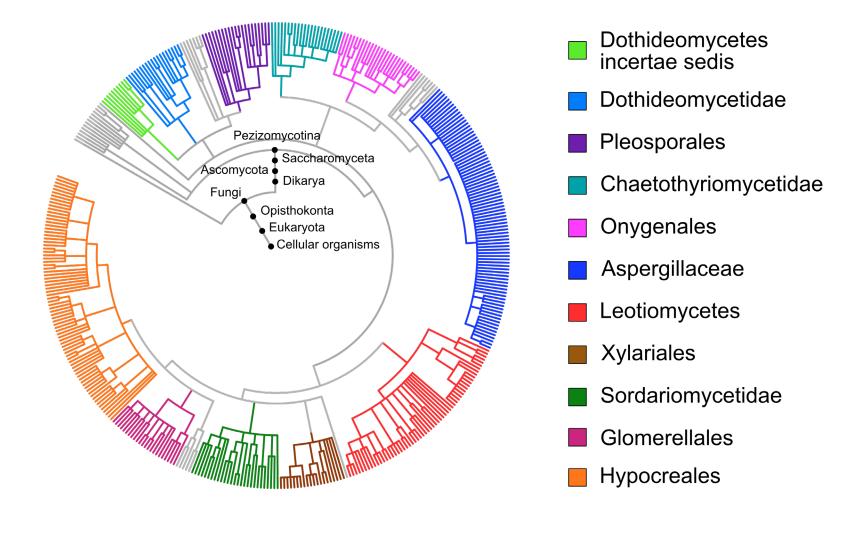




Entire MacCoss lab and Skyline team!

03/06/2024 15

Extra slides



18

