

Engineering of Methane Metabolism in *Pichia pastoris* through Methane Monooxygenase Expression

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Background

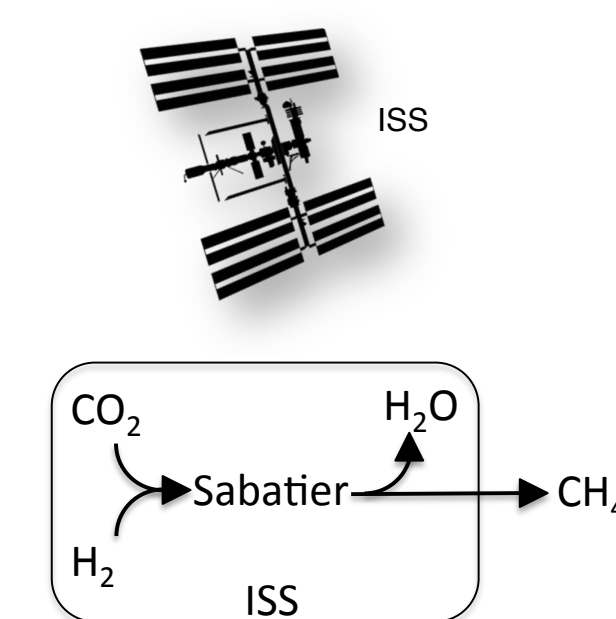
Utilization of available resources is important to minimize the need for costly resupply from Earth. Currently the oxygen reclaiming Sabatier system on the ISS reacts CO₂ and H₂ to form H₂O and CH₄. The water is recycled back into the ISS system, but the methane is vented into space as waste. One potential use for this methane is as a carbon substrate for a biological production platform such as the methylophilic yeast, *Pichia pastoris*. *P. pastoris* is a well-established synthetic biology platform and its native methanol metabolism is one enzymatic step away from metabolizing methane. In methanotrophic bacteria that step is carried out by methane monooxygenases (MMOs), which oxidize methane to methanol. In this project, we have attempted to engineer methane metabolism into *P. pastoris* by expressing a bacterial MMO system.

Sabatier produces methane

- The ISS Sabatier system produces methane and water from CO₂ and H₂.
- Water is recycled but CH₄ is vented to space.
- CH₄ could feed heterotrophic microbes for in-space bio-manufacturing.

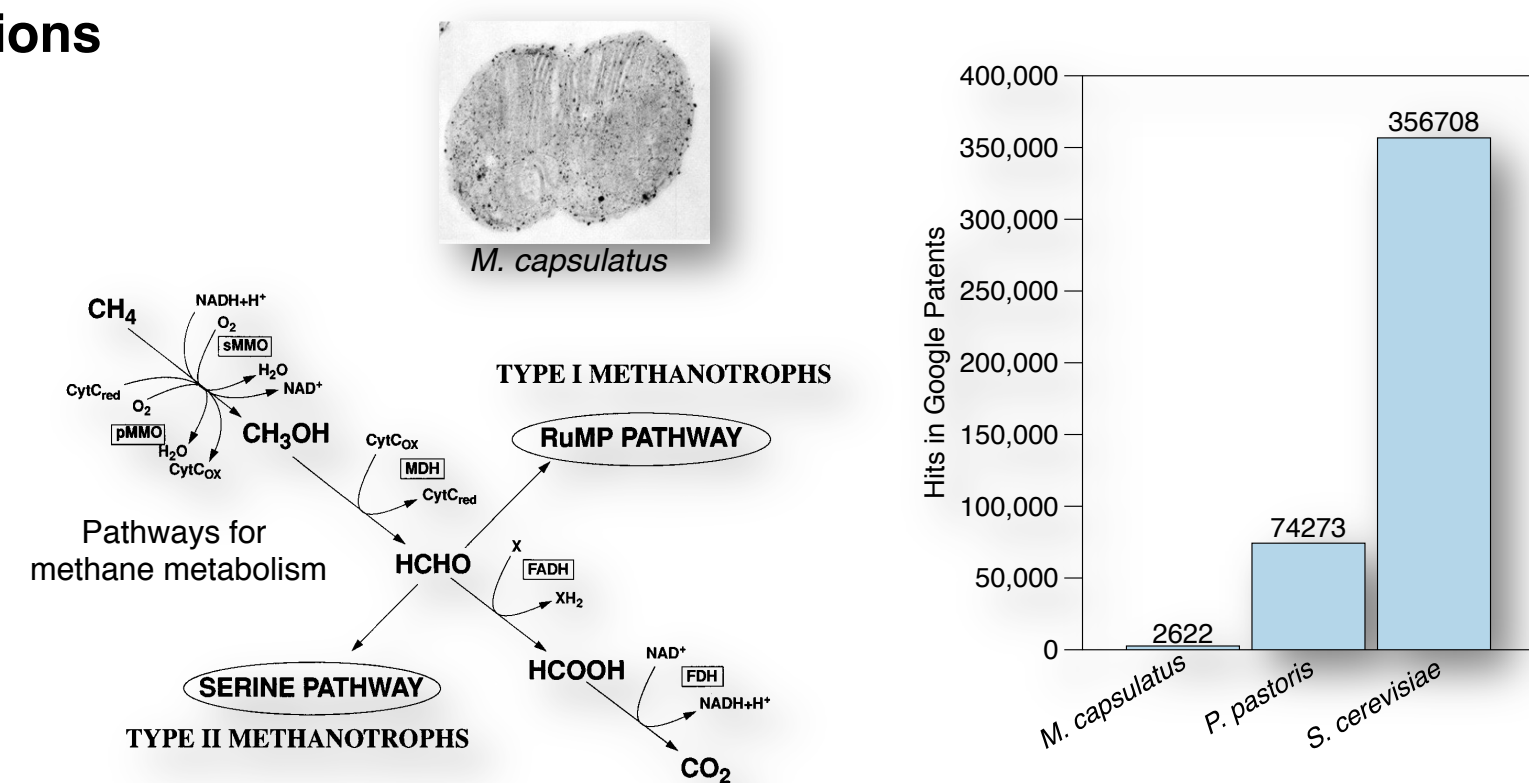


Astronaut installing Sabatier system



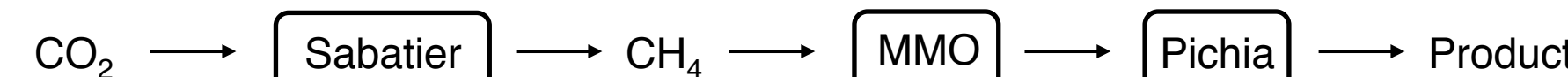
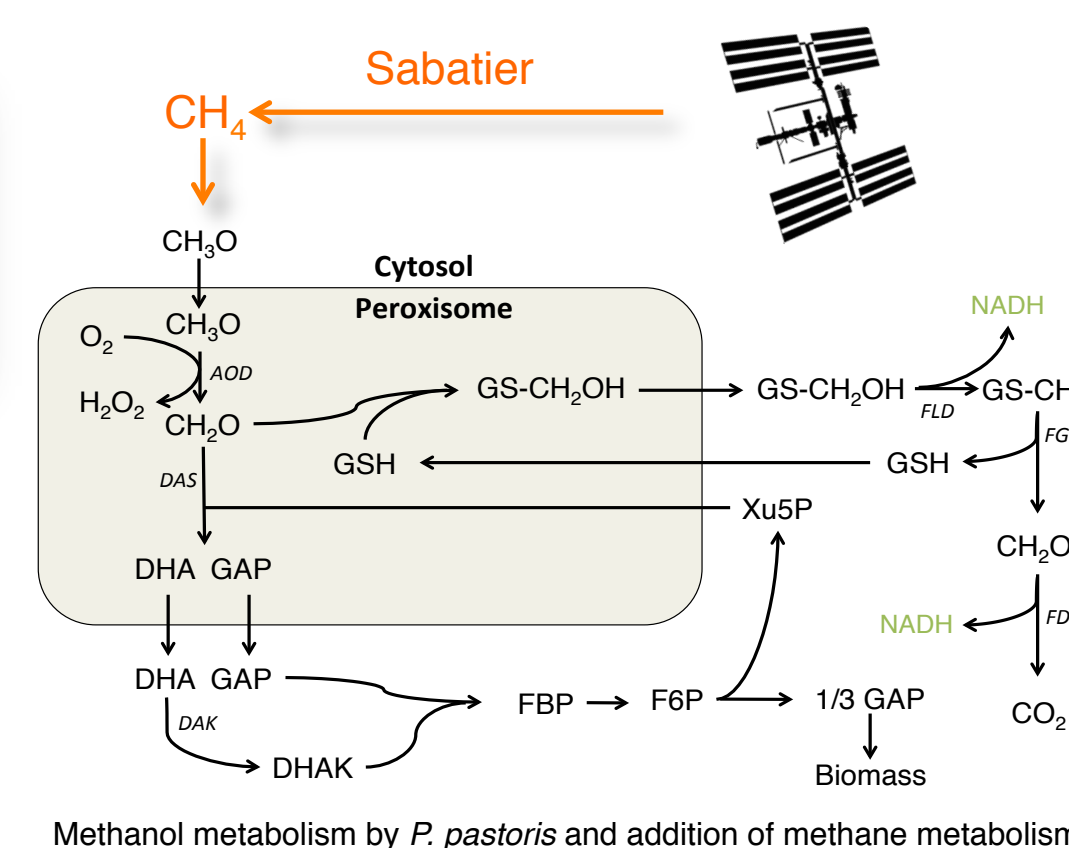
Natural methanotrophs have limitations

- Natural methanotrophic (consume methane) bacteria exist and are being developed as microbial factories.
- They utilize Methane Monooxygenases to hydroxylate methane to methanol.
- These microbes have limited engineering tools available and innovation is relatively slow.



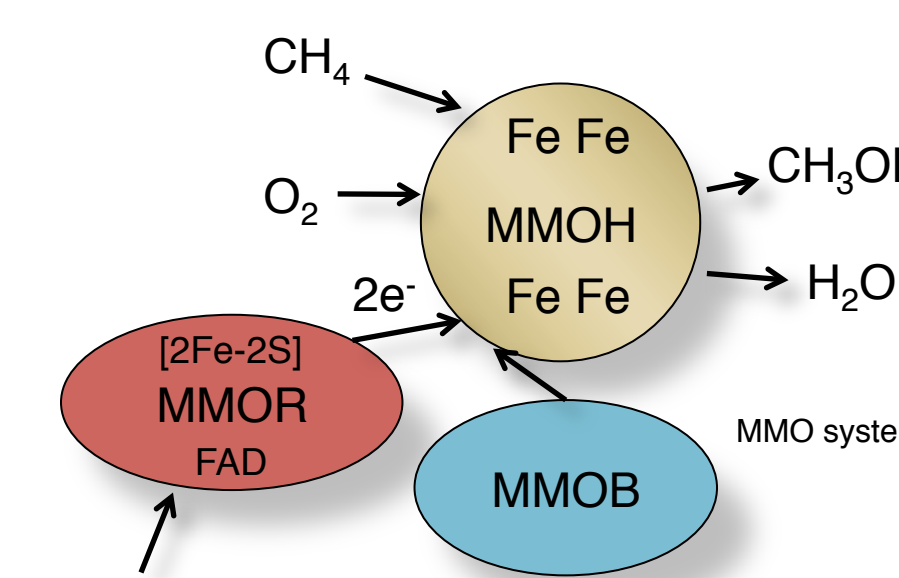
Pichia pastoris as a synthetic methanotrophic yeast

- P. pastoris* is well-established methylophilic (consumes methanol) yeast
- Used to produce Trypsin, murine TNF α , and FDA approved drugs Kalbitor and Jetrea.
- Addition of Methane Monooxygenase should allow growth on methane.

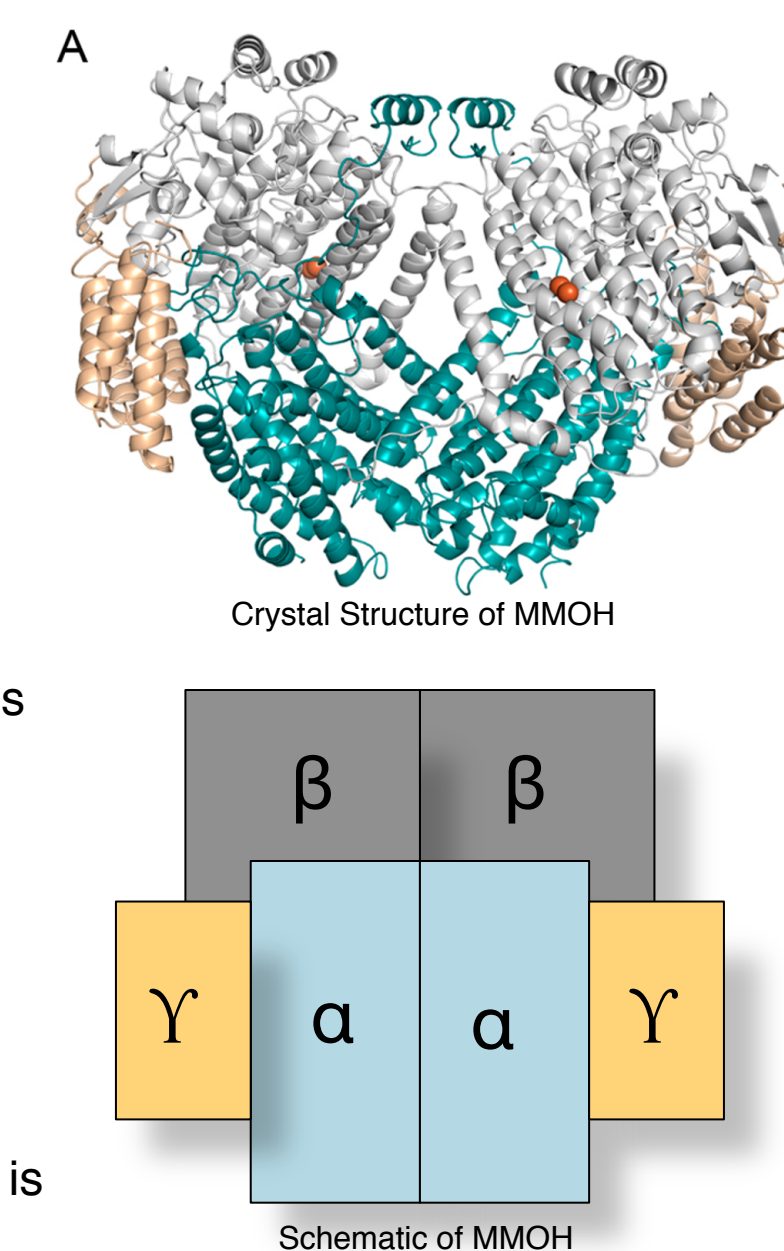


Results

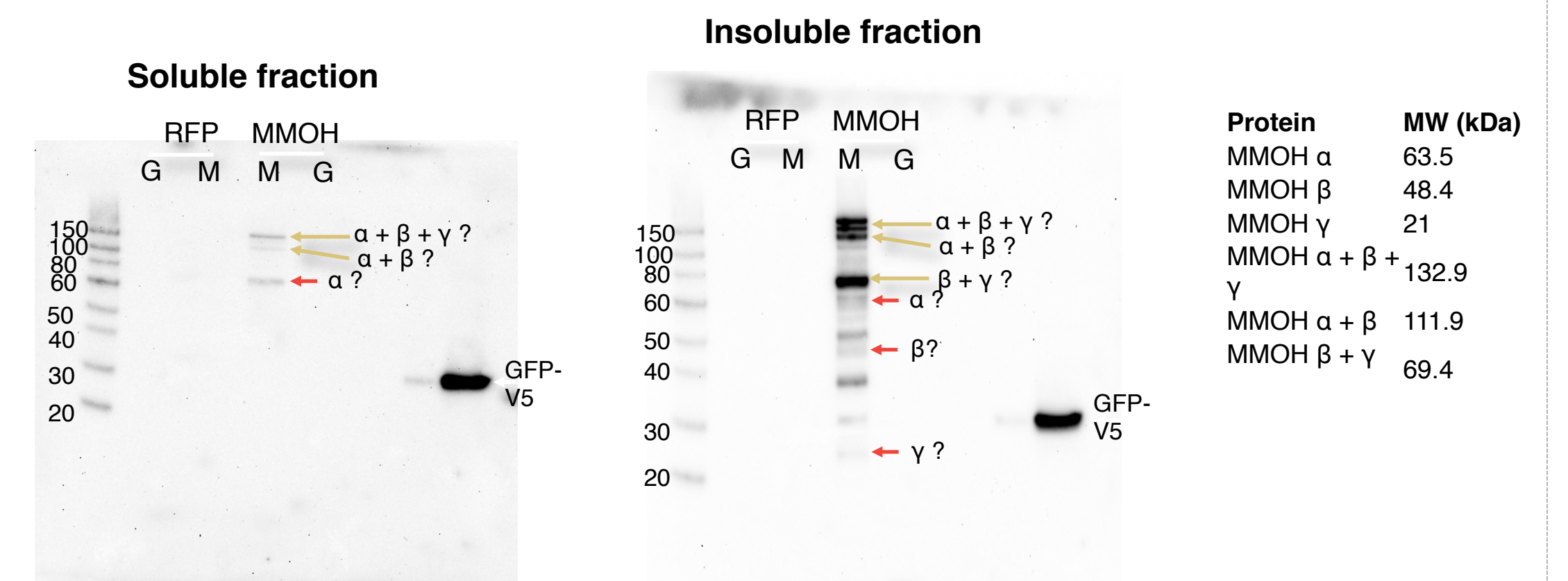
Methane monooxygenase system



- Methane hydroxylation in bacterial methanotrophs is carried out by MMOH, a hexamer of 3 polypeptides (α , β , γ).
- MMOR, a reductase, transfers electrons to MMOH and is also required.
- MMOB may be important to speed the catalytic cycle.
- Assembly of MMOH into the correct 3D structure is required and may not readily occur outside of native host.

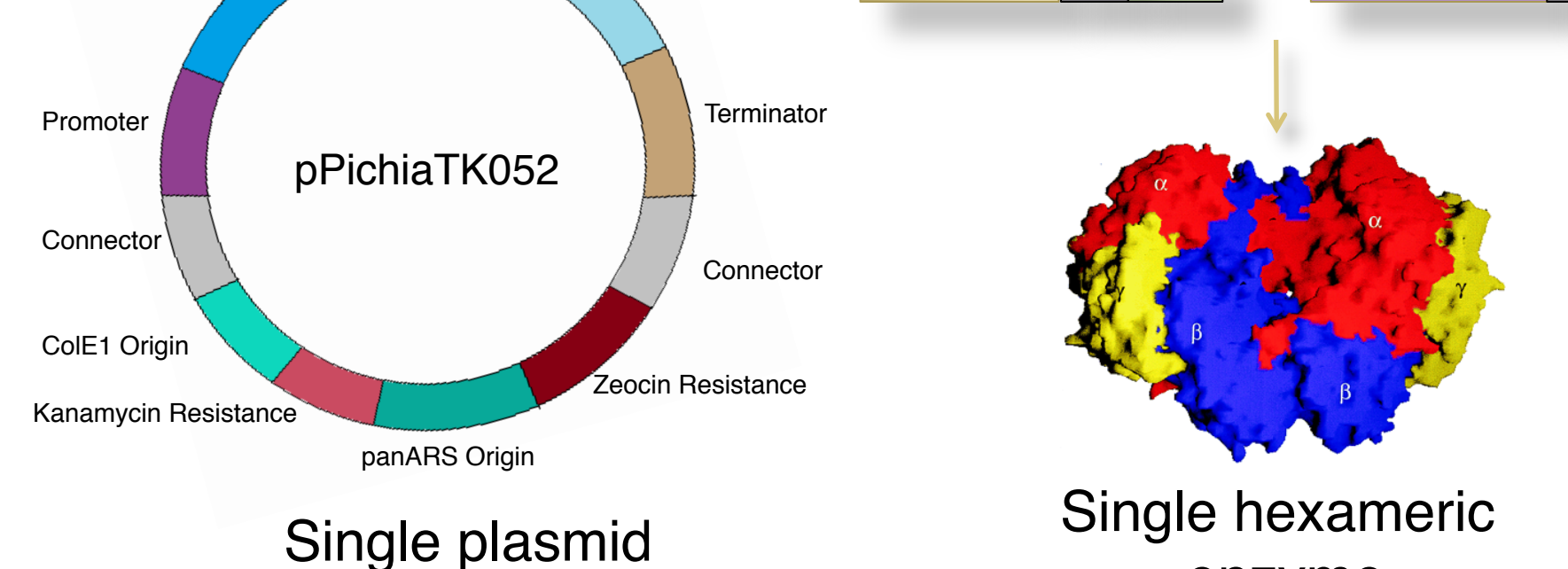
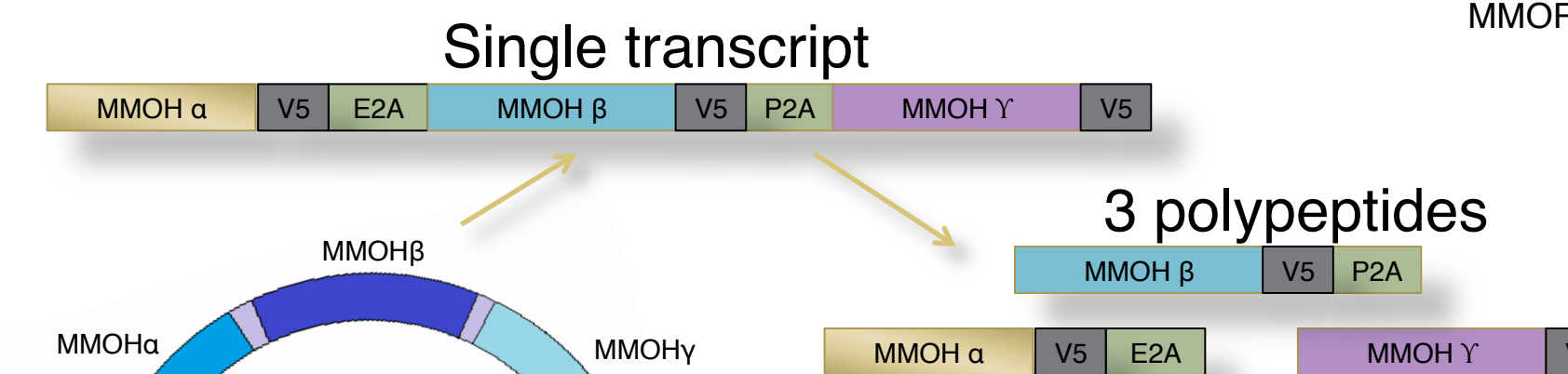
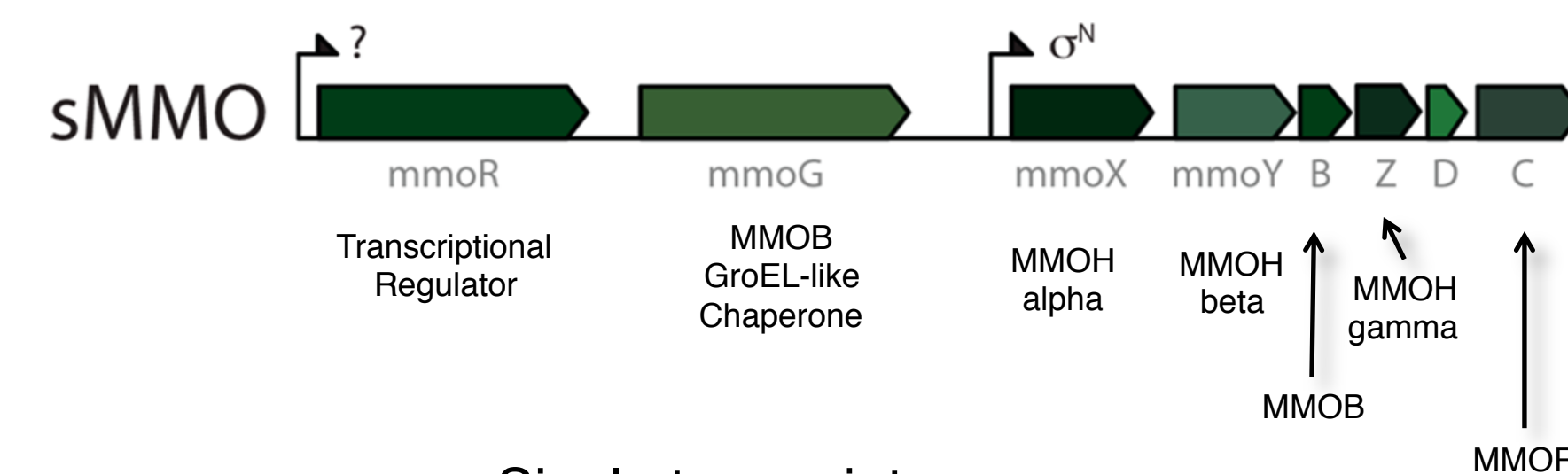


Methane monooxygenase expression



- Bands corresponding to MMOH subunits were found only in the lane from *P. pastoris* expressing the MMOH plasmid grown on methanol as expected for a gene expressed under a methanol induced promoter.
- Unexpected bands suggest incomplete skipping by type 2A peptides

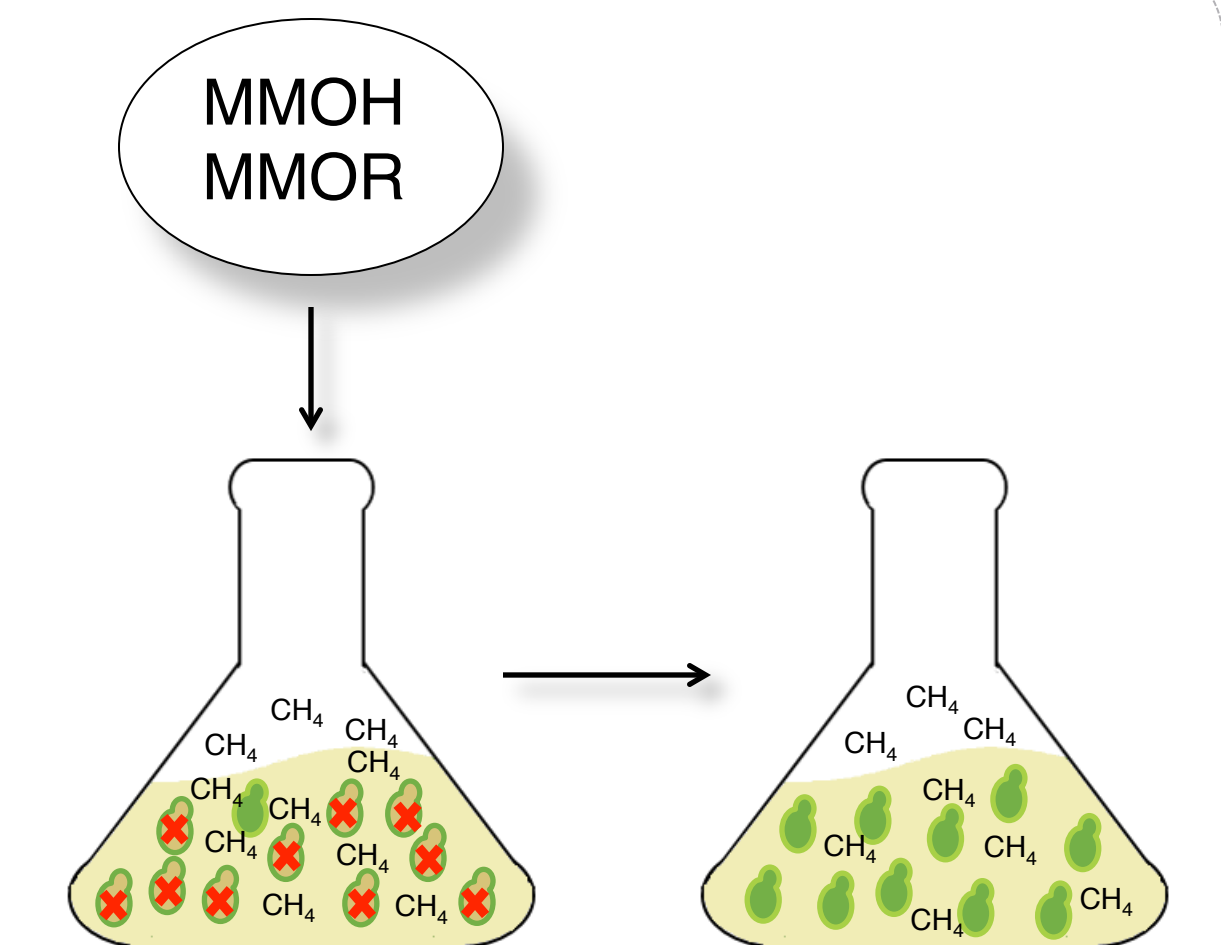
Balancing MMOH subunit concentration



- MMOH subunits are expressed from a single operon in methanotrophic bacteria suggesting that balanced subunit concentration is important
- To mimic this in *P. pastoris* we built plasmid where MMOH subunits are expressed on a single transcript that includes type 2A "skipping sequences".
- During translation of this transcript the ribosome should skip a peptide bond after the 2A sequences resulting in 3 polypeptides at equal concentration.

Testing minimal system

- Expression and assembly of MMOH is not optimal.
- Regardless, we have created a strain containing MMOH and MMOR, which contains all components necessary for *P. pastoris* growth on methane.
- We can now set up a powerful selection for a functional system by growing on strain in media containing methane as a sole carbon source.



Conclusions

Engineering *P. pastoris* to metabolize methane offers one way to utilize currently wasted methane. To engineer *P. pastoris* we have created new engineering tools including promoters to work in *P. pastoris* and shown that they are functional based on their ability to drive expression of RFP. Preliminary data suggests that *P. pastoris* is capable of expressing MMOH, but further testing needs to be done to confirm expression and functionality. While completing this testing we are also moving forward with engineering expression of other proteins in the MMO system, with the goal of ultimately growing engineered *P. pastoris* on a methane substrate for functional testing.

References

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